



The ProteinChip® Company

ProteinChip® Software 3.0 Operation Manual

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Getting started

This chapter briefly introduces the software, including data acquisition, calibration and analysis. More detailed descriptions of software functions can be found in the chapters that follow.

To get the most from this tutorial section, you should have a ProteinChip Array with a calibration sample prepared and ready to analyze with the ProteinChip Reader. In the example below, Ciphergen's All-in-1 Peptide Molecular Weight Standard (Cat # C100-0003) was used. (Instructions for preparing the Peptide Molecular Weight Standard sample are included with the product.)

Starting the software

1. Start the software by double-clicking its icon on the desktop, or by locating it through the Windows **Start** menu under **Programs | Ciphergen ProteinChip Software | Ciphergen ProteinChip Software 3.0**.



***NOTE:** If this is the first time the software has been started, the program will initialize the **Database Connection Wizard** to guide you through the steps of creating a new ProteinChip database folder (see Chapter 2, "Managing Data and Users").*

2. The startup screen will appear briefly, then the **ProteinChip Software Login** dialog will be displayed (Figure 1-1).

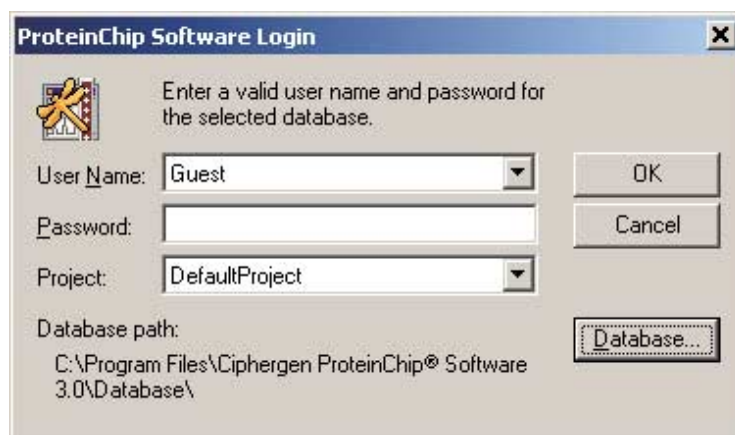


Figure 1-1: The **ProteinChip Software Login** dialog.

Select the appropriate **User Name**, enter the password (if any), and select the desired project from the drop-down list. You can also use the **Database** button to select a different database, or to create a new database using the database connection wizard (refer to "The database connection wizard" on pages 29–35 for detailed information on using the wizard). Confirm your selections by clicking **OK**.

The **Sample Exchange** dialog opens next (Figure 1-2). This dialog allows you to enter information about a ProteinChip Array before opening the sample port and inserting the chip.

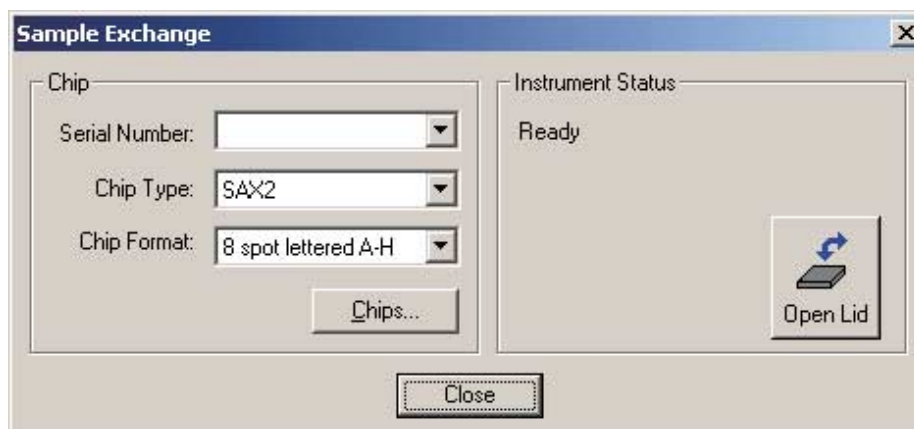


Figure 1-2: The **Sample Exchange** dialog.



IMPORTANT: The **Chip Format** field (i.e. 8 spot lettered A–H, the default selection) is used to position the sample spot inside the instrument, so be careful to select the correct format. The **Serial Number** and **Chip Type** fields are optional.

Inserting a ProteinChip Array into the reader

To insert a ProteinChip Array into the instrument,

1. Click the **Open Lid** button in the **Sample Exchange** dialog to initiate the lid opening sequence. After a short delay, the green LED next to the sample lid will light up and the automatic lid will open. (If using the model PBS I reader, manually open the lid.)
2. If there is a ProteinChip Array in the instrument, remove it.
3. Insert the ProteinChip Array into the slot on the sample port. Make sure the CIPHERGEN logo is at the top (the spot “A” end of arrays without the logo) and the spots are facing the back of the instrument.
4. Click the **Close Lid** button in the **Sample Exchange** dialog. (If using the model PBS I reader, manually close the lid.)
5. The **Select Current Chip** dialog (Figure 1-3) will open on top of the **Sample Exchange** dialog. This dialog allows you to enter data about the individual samples on the chip, and is particularly useful for keeping track of profiling, and other high-sample-number experiments. Data entry in this dialog is optional. To close the dialog, click **OK** to accept the information you entered, or **Cancel** to exit the dialog without saving any changes. (You can open the **Select Current Chip** dialog later to edit or add information.)

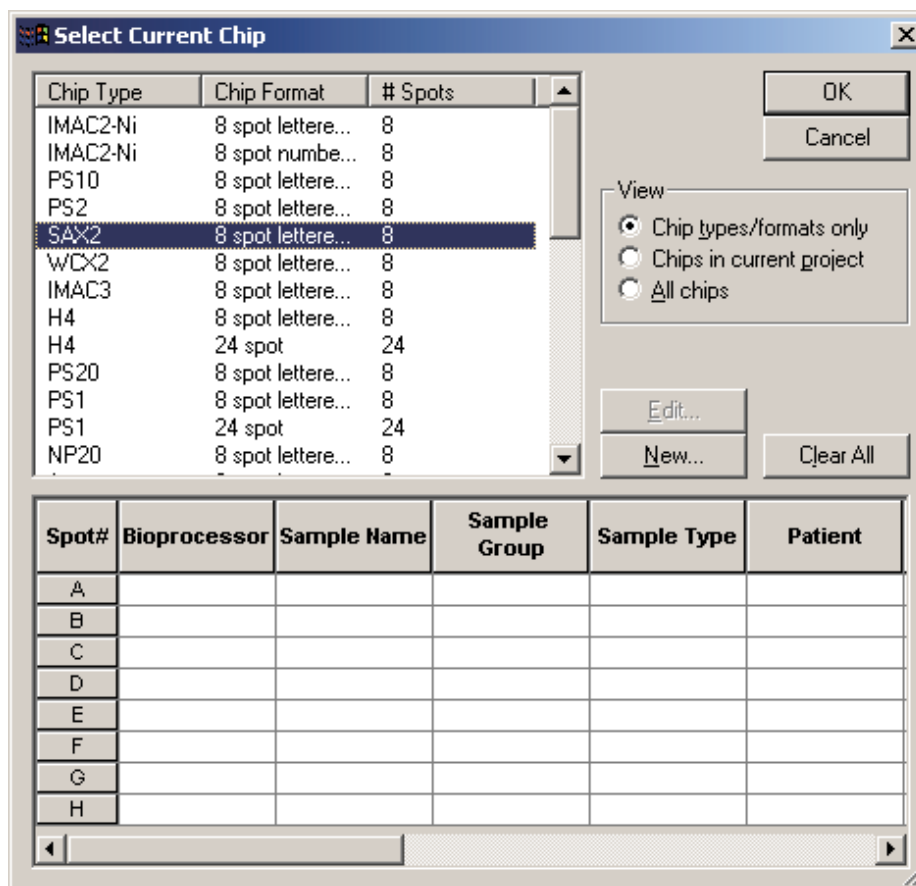


Figure 1-3: The *Select Current Chip* dialog.

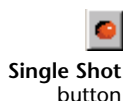
- The **Sample Exchange** dialog (which remained open beneath the **Select Current Chip** dialog) will display the instrument status as the sample exchange progresses. You can close the **Sample Exchange** dialog by clicking **Close** any time after the lid is closed. After 1 to 5 minutes, the instrument will be ready to begin acquiring data.

Data acquisition

If you're using the All-in-1 Peptide Standard, enter the initial instrument settings values given in the table below into the appropriate toolbars or into the **Manual Protocol** dialog (see "Manual protocols" on page 9).

Starting Conditions	PBS I	PBS II
Laser Intensity	10	170
Detector Sensitivity	9	9
NDF Filter	IN	N/A
High Mass	10000	10000
Start Optimization Range	1000	1000
End Optimization Range	9000	9000

Table 1-1: Recommended initial instrument settings for use with the All-in-1 Peptide Standard.



Fire the laser by clicking the **Single Shot** toolbar button. Individual shots are displayed in the **Most Recent Shot** window (Figure 1-4). If the window isn't open, choose **View | Most Recent Shot** to display it.

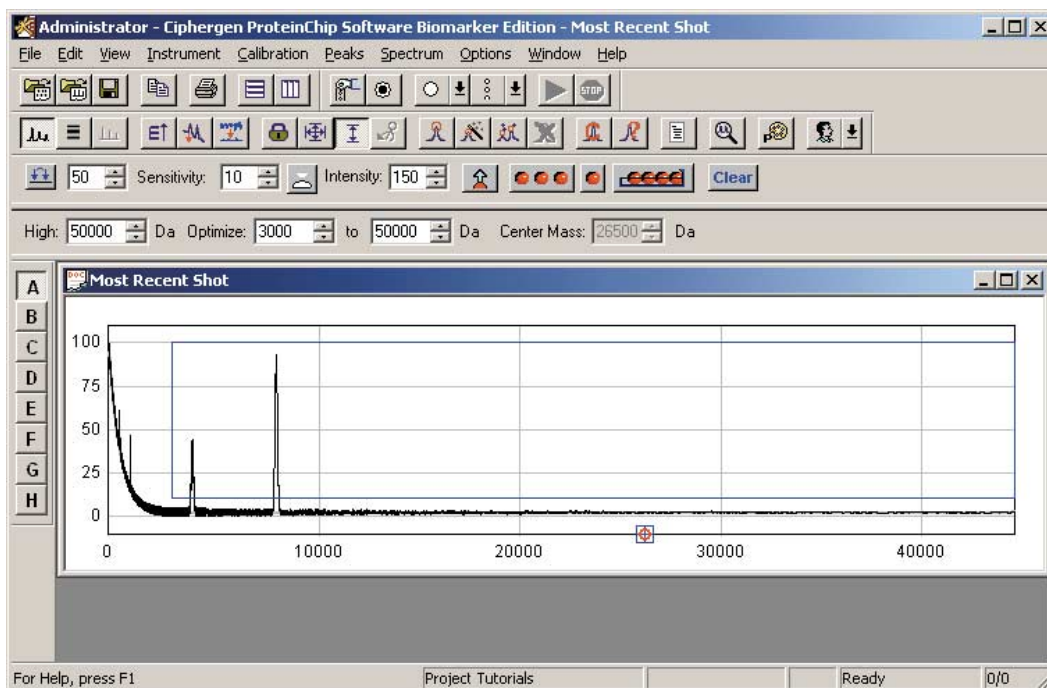


Figure 1-4: Sample single shot data.

Making adjustments

If the peaks of interest are at or near 100 on the vertical scale, reduce the sensitivity or the laser intensity. If the peaks are very small, increase the laser intensity.

Review the spectra in Figure 1-5 for examples of what to look for when making adjustments.

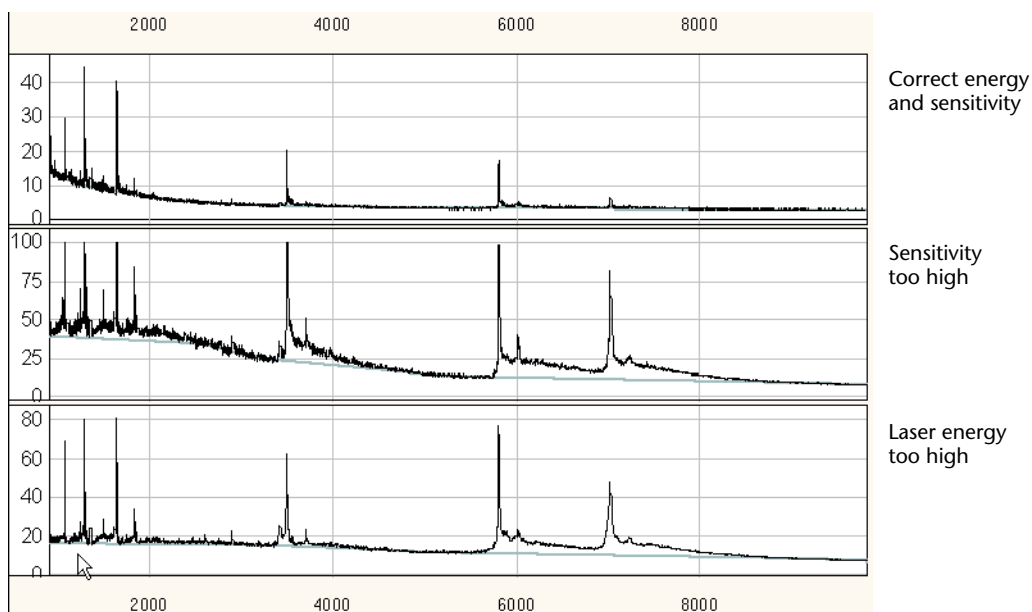


Figure 1-5: Sample spectra.

- The top spectrum in Figure 1-5 represents appropriate laser energy and detector sensitivity settings. The peaks of interest (~3500 MW to ~7000 MW) are about 20% full scale, and the baseline noise is minimal.
- The middle spectrum shows a much noisier baseline. To increase the signal-to-noise ratio, decrease the sensitivity setting.
- The bottom spectrum has a smoother baseline than the middle spectrum, indicating an appropriate sensitivity setting. However, better quality data would result from using a lower laser intensity.


Fire Continuously
button


Average
button


Fresh Position
button

Once the approximate settings are found, click the **Fire Continuously** button, then the **Data Average** button. Data will begin to accumulate in the **Data Average** window (Figure 1-6). As more data are incorporated into the average, the trace will become smoother and the peaks more sharply defined. Collect at least 10 shots to see the improvement in signal-to-noise that averaging the signal provides.

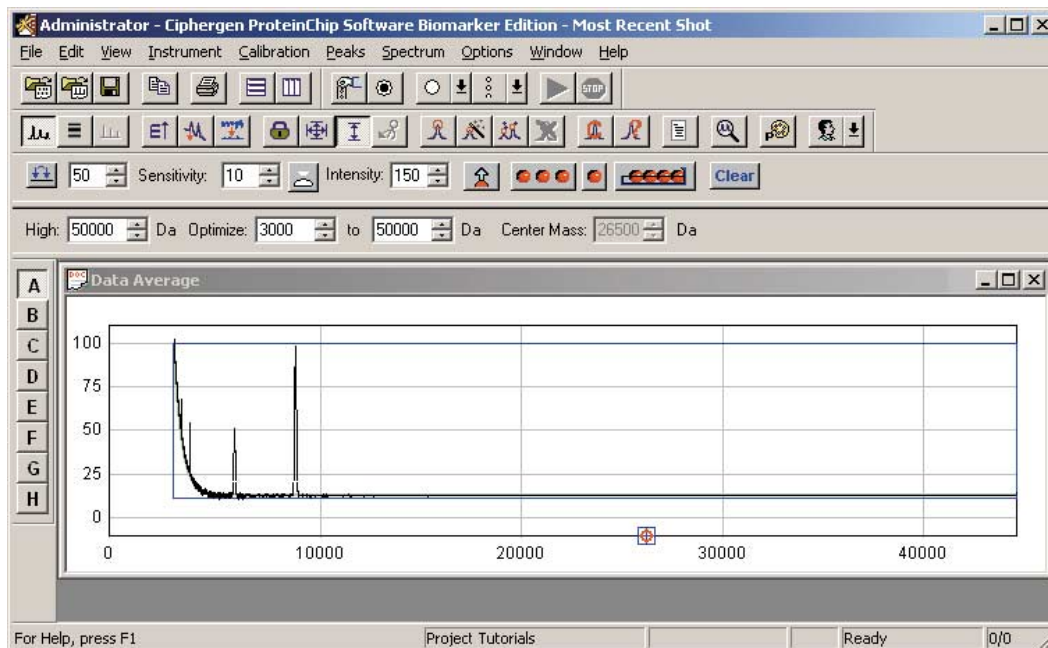


Figure 1-6: Sample averaged data.

After many repeated shots in the same spot position, the sample may become depleted, evidenced by dramatically reduced signal strength in the **Most Recent Shot** window. Click the **Fresh Position** button to move to a new position. Click the **Fire Continuously** button a second time to stop firing the laser.

Manual data collection

Manual data collection is useful when the optimal mass ranges, laser intensity or detector sensitivity must be established. Before you begin to collect data, you must specify certain instrument parameters. These include the individual spot to be analyzed (1–8, 1–24 or A–H depending on the chip format; selected via the **Sample Exchange** dialog), the mass range to be collected, lag time setting, laser position within each spot, detector sensitivity, and laser intensity. The settings can be entered directly in the **Direct Control** and **Data Acquisition** toolbars or entered into a manual protocol. The previous section, “Data acquisition” on page 6 shows an example of entering the instrument parameters directly. See “Manual protocols”, below, for information on creating and using manual protocols.

Manual protocols



Manual Protocol button

Creating and editing manual protocols

Manual protocols allow convenient storage and recall of sets of instrument conditions for manual data collection. They are created and edited in the **Manual Protocol Properties** dialog (Figure 1-7). The dialog is accessed by choosing **Manual Protocol** from the **Instrument** menu, or by clicking the **Manual Protocol** button in the **Data Acquisition** toolbar.

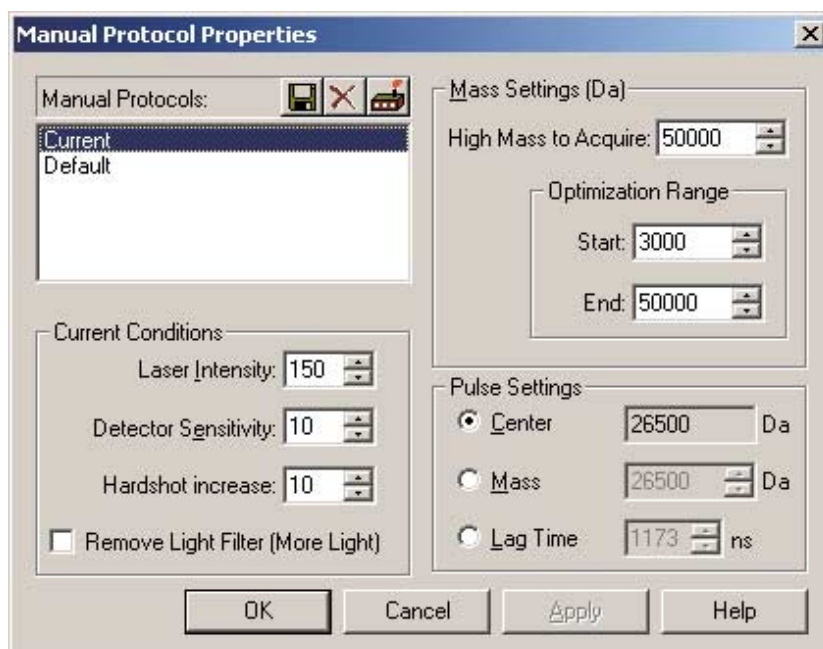


Figure 1-7: The *Manual Protocol Properties* dialog.

The functions of the buttons and fields in the **Manual Protocol Properties** dialog are as follows:

- **Manual Protocols:** this list is used to select manual protocols. The values in the dialog change to reflect the protocol selected from the list.
- **Save As** (small disk picture): opens a dialog that allows you to name and save the protocol.
- **Delete** (X): deletes the protocol selected in the **Manual Protocols** list.
- **Factory Defaults** (small picture of a factory): resets the protocol to the factory default settings.

Current Conditions settings

- **Laser Intensity:** controls the current laser intensity. The range for the model PBS I reader is 1–100, for the model PBS II reader it is 0–300. A useful laser intensity to begin with is 100. Increase or decrease the laser intensity until the peaks of interest are between 10 and 90 percent of full scale.

- **Detector Sensitivity:** changes the gain of the digitizer. Increase the sensitivity to increase the size of peaks, decrease it if the peaks of interest are off scale. The detector sensitivity range is 1 to 10, with 10 being the most sensitive. A detector sensitivity of 10 is routinely used. If the peaks can't be adjusted to stay on scale, then lower the sensitivity until they are.
- **Hardshot Increase:** controls how many units of laser energy to add when the **Hard Shot** button is clicked.
- **Remove Light Filter:** controls whether the NDF is in or out of the optical path (model PBS I reader only).

Mass settings

- **High Mass to Acquire:** this value sets the largest mass that will be collected. Larger mass ranges require the collection and analysis of more data points, thus slowing data acquisition and processing speed. The upper mass limit for the instrument is 500,000 Da.
- **Optimization Range:** this range defines the region for the global signal to noise calculation. It should be set large enough to include any protein masses you are expecting to detect. The highest intensity peak in the region will be used for the signal to noise value. The model PBS II reader also uses this range for time lag focusing. If the time lag focusing (discussed below) is referenced against the optimization range, time lag focusing will be automatically set to the center of the optimization range you have defined.

Pulse (Time Lag Focus) settings

Time lag focusing (available only on the model PBS II reader) improves peak resolution by delaying the time between the laser pulse striking the sample and the time an extraction pulse is applied to accelerate the ionized proteins. The time between the laser pulse and the extraction pulse is called lag time. Its immediate benefits are increased resolution, particularly in the low mass range.

The pulse setting defines how the time lag focus will be determined:

- **Center:** this option automatically calculates the lag time based on the center of the optimization range.
- **Mass:** this option defines the lag time directly, based on mass.
- **Lag Time:** this option allows the user to define the lag time directly, based on time (in nanoseconds).

The optimum time lag focus for protein resolution changes depending upon the protein's mass. No single focus point will be optimal for all mass ranges, so we suggest choosing a time lag focus for particular mass ranges using the following criteria. To set a general time lag focus, base it on the optimization range. Selecting an optimization range (see above) that encompasses the mass range you are interested in will ensure a generally optimized spectra. If you find a protein of interest, or you already know the protein you are interested in, select the focus based on **Mass**. This will give

the highest resolution for a particular mass. As a third alternative, if you know the relationships between ion cloud densities and the particular accelerating potentials of your instrument, you *can* set the time lag focus based on time (in nanoseconds), but the other two methods give the same result and are just easier!

To generate the highest quality of data, it is important to select an appropriate focus range. A focus mass that is greatly different from the mass range you are interested in will result in broad, jagged peaks that are poorly resolved.

Saving manual protocols

After you have finished making all desired settings in the **Manual Protocol Properties** dialog, click the **Save** (disk) button in the upper left side of the dialog to assign a name to the new protocol, then save it. It will be added to the list of protocols in the **Manual Protocol Properties** dialog.

Managing manual protocols

Manual protocols reside in the **Manual** subfolder in the user's folder inside the **Ciphergen ProteinChip Software 3.0** folder (accessible via the **Windows Explorer**, Figure 1-8). Like all other protocols, manual protocols can be shared with other users by copying the protocol into that user's **Manual** subfolder.

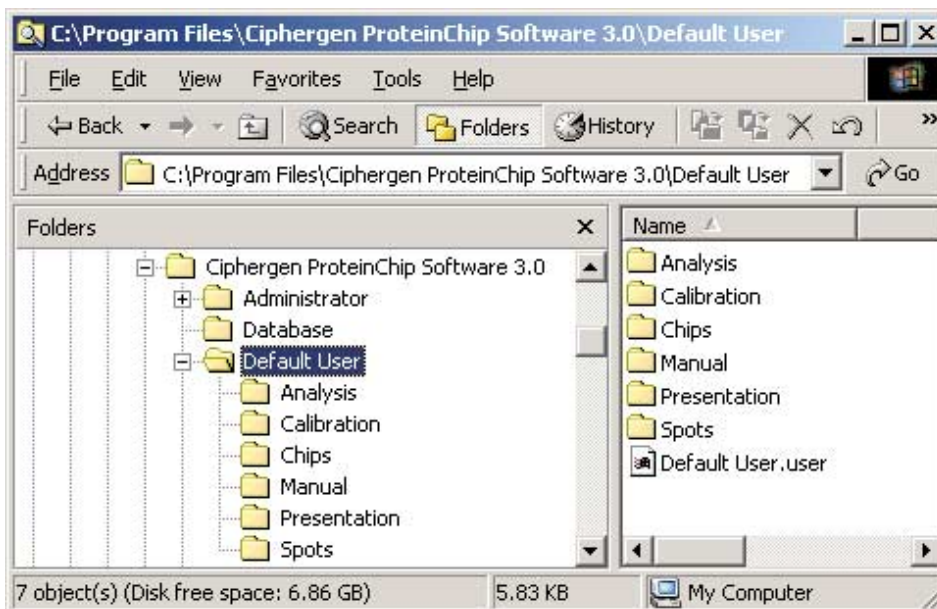


Figure 1-8: *Windows Explorer* view of the contents of the **Ciphergen ProteinChip Software 3.0** folder. The sub-folders within the user's folder are labelled with the type of protocol they contain.

Protocols can be transferred, shared, or copied between users with no restrictions. To delete a protocol, use **Windows Explorer** to remove the protocol from its subfolder.

Automatic data collection

Automatic data collection is designed to analyze a number of spots on a ProteinChip array under a pre-defined set of conditions. Data can be collected using static conditions, in which the data acquisition settings are identical for all spots on the chip, or dynamic conditions, in which laser intensity is adjusted automatically to optimize for a particular mass range. Static conditions are generally used for quantitation.

Automatic data collection has two main components: spot protocols, and chip protocols. A spot protocol contains instructions to control data collection for a spot. A chip protocol is essentially a sequence of spot protocols.

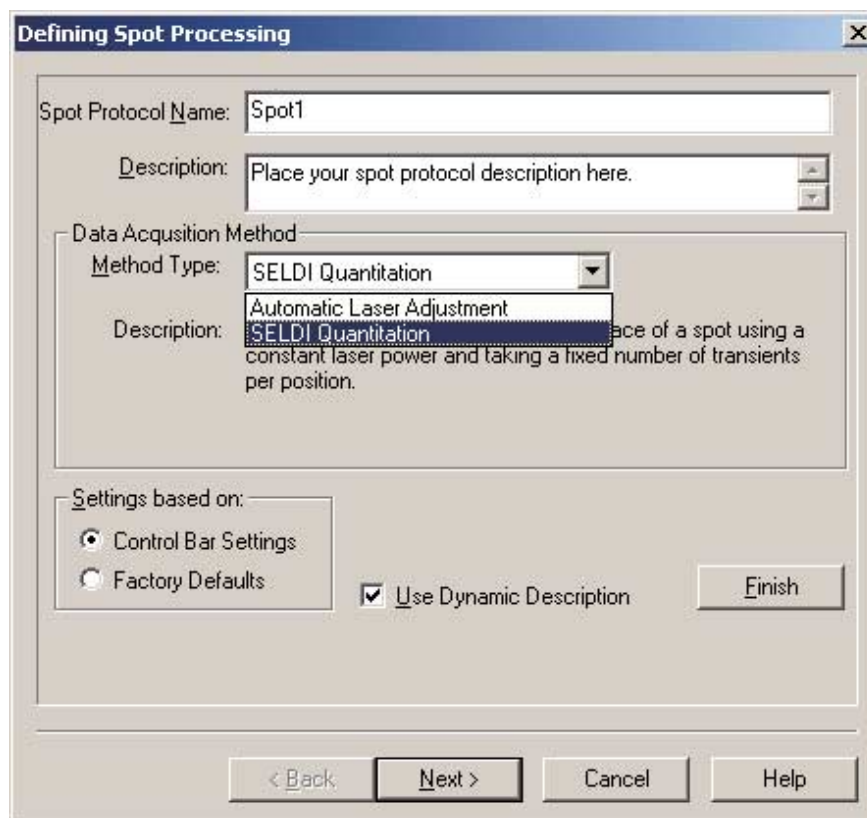
Spot protocols

Spot protocols provide instructions to the instrument for automatic data collection from a single sample spot. The settings define the mass range, laser intensity control, and other parameters, which vary according to spot protocol type.

Creating and editing spot protocols



Select **Spot Protocol** from the **New** submenu of the **File** menu, or press the **Spot Protocol** toolbar button to open the spot protocol wizard (Figure 1-9).



Defining Spot Processing

Spot Protocol Name: Spot1

Description: Place your spot protocol description here.

Data Acquisition Method

Method Type: SELDI Quantitation

Description: Automatic Laser Adjustment

Settings based on:

☒ Control Bar Settings

☐ Factory Defaults

☒ Use Dynamic Description

Finish

< Back Next > Cancel Help

Figure 1-9: The first page of the spot protocol wizard.

The first page of the spot protocol wizard (the **Defining Spot Processing** page) gathers general information about the spot protocol that you are creating. Enter a unique name for the protocol in the **Spot Protocol Name** field, and any desired descriptive text in the **Description** field.

The most important value chosen on this page is the **Data Acquisition Method**. There are two method types, *Automatic Laser Adjustment* and *SELDI Quantitation*.

Automatic Laser Adjustment data acquisition method

This method adjusts the laser intensity during collection and is suitable for collecting data for protein identification and characterization, in cases where you know the existence of a protein in a narrow mass and intensity range (defined as the optimization range). The optimization range is used to determine which transients are included in the data average. The software actively adjusts the laser intensity and data collection to obtain the best spectra within that optimization range.

SELDI Quantitation data acquisition method

Select this method when collecting data for comparison. The method maintains constant laser intensity and collects a specified number of transients at multiple sample positions to create a representative average of the sample.

The **Settings based on** panel allows you to select the analysis settings. The available options are the **Current Control Bar** settings or the **Factory Defaults**.

To review or change specific parameters directly from the keyboard, click the **Next** button. This will take you to the first of several **Auto Setup** pages. The first page defines the **High Mass to Acquire** and the **Optimization Range**.

Figure 1-10: The Auto Setup page of the spot protocol wizard.

The optimization range should be set according to whether **Automatic Laser Adjust** or **SELDI Quantitation** method of data acquisition was selected in the previous window. If **Automatic Laser Adjust** is the method of data acquisition, select a narrow mass range that flanks either side of the known mass for which you are optimizing. If **SELDI Quantitation** is the method of data acquisition, define an optimization range that will include all masses of interest. If the time lag focusing is based on the optimization range, a time lag focus value corresponding to the center of the optimization range will be automatically entered. Enter the starting laser and detector sensitivity values. In the **Focus By** section, select the appropriate value. When finished, click the **Next** button.

If you are using the **Automatic Laser Adjust** method of acquiring data, the **Auto Laser Definition** window will be the third window in the spot protocol wizard (Figure 1-11). Enter the total number of laser shots to collect in the **Data Average** window, typically 50–100. Define the on-scale intensity and off-scale intensity optimization range, typically 10% (value = 26) to 80% (value = 204). Next define how the laser is adjusted, i.e. “Increase by 4 after 3 consecutive LOW shots” and “Decrease by 1 after 2 consecutive HIGH shots”. Click **Finish** when done.

Auto Laser Method Definition

Shots to collect: 50

Thresholds

Points on-scale to ACCEPT: 3 On-scale intensity: 51 20.0%

Points off-scale to REJECT: 2 Off-scale intensity: 242 94.9%

Laser Adjustments

Increase by 1 after 1 consecutive LOW shots.

Decrease by 3 after 1 consecutive HIGH shots.

☒ Revive signal with increased laser intensity:

8 consecutive shots w/out signal, boost intensity by 10

Position Changes

Minimum number of shots taken per position: 2

Maximum number of shots kept per position: 10

< Back Finish Cancel Help

Figure 1-11: The Auto Laser Method Definition page of the spot protocol wizard.

If you are using the **SELDI Quantitation** method of acquiring data, the **SELDI Data Acquisition Settings** page will be the third page in the spot protocol wizard (Figure 1-12). In this page, you define how the laser will move across the spot. Each spot can be accessed through 100 different positions (position 1 is at the bottom of the spot, position 100 is at the top of the spot). Define the **Starting at position** and **Ending at position** (typically starting at 20 and ending at 80). Next define the interval to move between the starting position and ending position, typically 5. Finally, define how many transients should be collected and averaged for each position, typically 5–10. Often the quality of spectra can be improved by warming the spot first. Warming a spot includes firing the laser, typically 2–5 times, at high intensity before averaging transients into the spectrum file. Generally, you should not include warming transients in a spectra file. Click **Finish** when done.

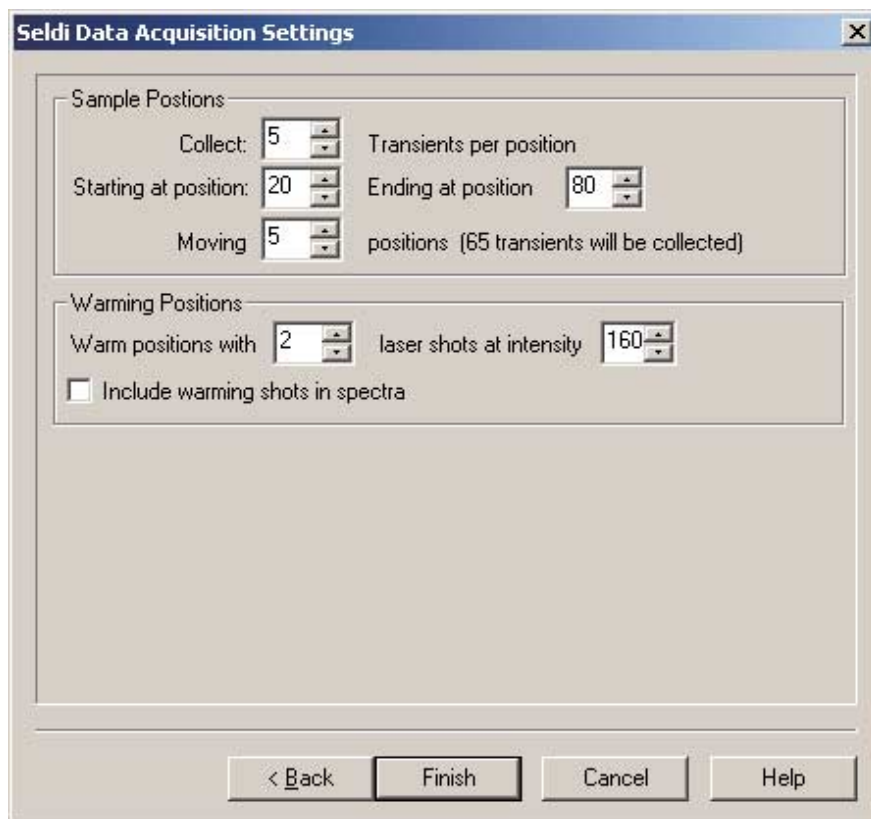


Figure 1-12: The SELDI Data Acquisition Settings page of the spot protocol wizard.

When the spot protocol wizard has finished, the spot protocol will be displayed.

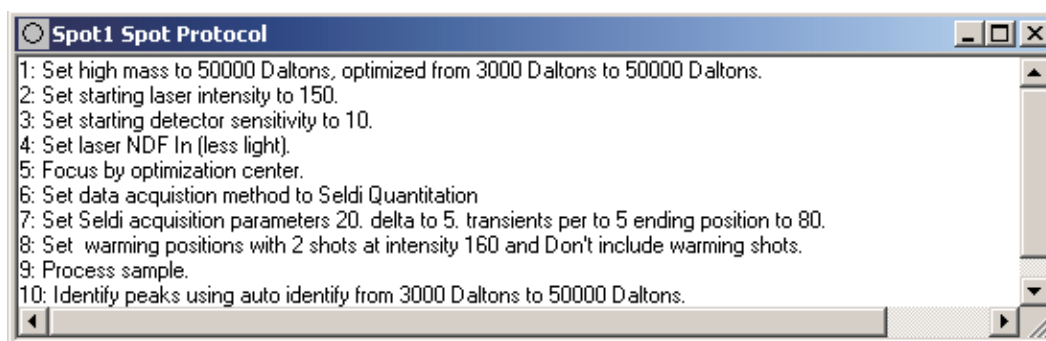


Figure 1-13: A completed spot protocol.

- To change the values in a line in the spot protocol, double-click the line you wish to edit, and the **Modify Command** dialog will appear (Figure 1-14), enabling you to change the command or associated settings. Delete com-

mands by selecting them, then pressing <Delete> or by selecting **Delete** from the **Edit** menu. New commands can be inserted above the selected line by pressing the <Insert> key or by selecting **Insert** from the **Edit** menu.

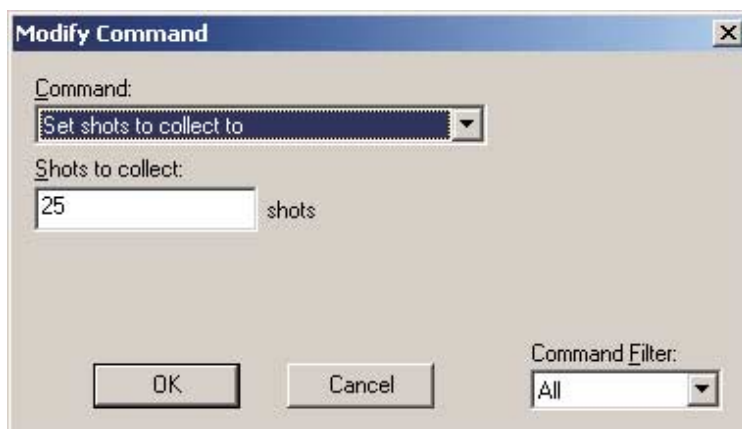


Figure 1-14: The **Modify Command** dialog.



- To open an existing spot protocol, click the arrow next to the **Spot Protocol** toolbar button and select the protocol from the drop-down list.
- To run a spot protocol, activate the **Spot Protocol** window and click the **Start Running** button in the **Standard** toolbar, or choose **Execute Spot Protocol** from the **Instrument** menu.



NOTE: If you make any changes to a spot protocol, you must save the protocol file (either under the same name or a different name) before the changes will be reflected when an automatic analysis run is started.

Managing spot protocols

Spot protocols reside in the **Spot** subfolder in the user's folder inside the **Ciphergen ProteinChip Software 3.0** folder (accessible via the **Windows Explorer**, Figure 1-15). Like all other protocols, spot protocols can be shared with other users by copying the protocol into that user's **Spot** subfolder.

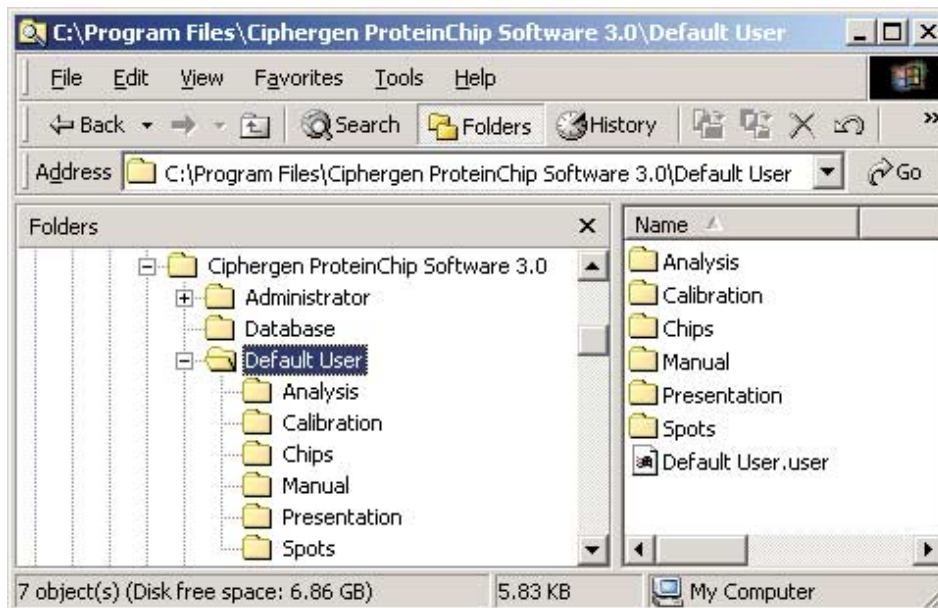


Figure 1-15: *Windows Explorer* view of the contents of the **Ciphergen ProteinChip Software 3.0** folder. The sub-folders within the user's folder are labelled with the type of protocol they contain.

Protocols can be transferred, shared, or copied between users with no restrictions. To delete a protocol, use **Windows Explorer** to remove the protocol from its subfolder.

Chip protocols

A chip protocol is a collection of instructions used to automatically collect data from a series of spots on a ProteinChip Array. Chip protocols are specific to a particular array configuration. A chip protocol consists of one line for each data collection on each spot.

Creating and editing chip protocols



To create a new chip protocol, select **Chip Protocol** from the **New** submenu of the **File** menu, or press the **Chip Protocol** toolbar button to open the chip protocol wizard. The first page of the wizard is the **Selecting Spots on Chip to Process** page (Figure 1-16).

Figure 1-16: The first page of the chip protocol wizard. Note that the **for Run** field will not be displayed unless the **Total Chip Runs** value is >1.

- **Chip Protocol Name:** assign a unique name to the chip protocol.
- **Chip Configuration:** select the chip format.
- **Spot Protocol:** select the spot protocol to use from the drop-down menu. By default, the wizard creates a chip protocol that uses the same spot protocol for all spots in a run. Once the chip protocol has been created, it can be modified to apply different spot protocols to individual spots.
- **Total Chip Runs:** this value determines how many times the spots will be analyzed. When **Total Chip Runs** is greater than 1, the **for Run** field will appear directly below the **Total Chip Runs** field. To analyze spots more than once using different spot protocols:
 1. Set the number of runs in **Total Chip Runs**.
 2. Select the spot protocol from the **Spot Protocol** combo-box.
 3. Change the **for Run** value and repeat steps 1 and 2 for the remaining runs.
- **Increment:** sets data collection from all or some spots.
- **First Spot** and **Last Spot:** control the range of spots to process.
- **Process Order:** selects the order in which the spots will be run when multiple runs are used. The choices are **Spot**, which repeats the spot for the number of runs, before advancing to the next spot; and **Run**, which advances from spot to spot, repeating the advances for the number of runs requested.

After selecting the spots to process, click the **Next** button to move to the **Selecting Method of Spot Naming** page (Figure 1-17).



Figure 1-17: The second page of the chip protocol wizard.

Enter a short description of the protocol if desired. If the **Use Dynamic Description** box at the bottom of the window is unchecked, the contents of the description you have entered will be displayed in all menus beside the chip protocol name. If the **Use Dynamic Description** box at the bottom of the window is checked, information regarding specific parameter settings will be displayed in all menus beside the chip protocol name.

Determine how each spot will be named in the **Auto-naming of Spots** section. If you select **Root Name**, the **Root Name** field will become available to enter a common phrase from which all spot names will be built.

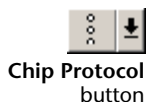
Click **Finish** when done.

When complete, the protocol is displayed as a summary diagram of all spots, the name of each spot and the spot protocol that will be used (Figure 1-18).

Chip1 Chip Protocol				
	Spot	Spectrum Tag	Spot Protocol	Spectrum Comment
<input type="radio"/>	A	Chip1-A	Spot1	
<input type="radio"/>	B	Chip1-B	Spot1	
<input type="radio"/>	C	Chip1-C	Spot1	
<input type="radio"/>	D	Chip1-D	Spot1	
<input type="radio"/>	E	Chip1-E	Spot1	
<input type="radio"/>	F	Chip1-F	Spot1	
<input type="radio"/>	G	Chip1-G	Spot1	
<input type="radio"/>	H	Chip1-H	Spot1	

Figure 1-18: A sample chip protocol.

- Spot processing order can be changed by clicking on the spot position indicator in the **Spectrum Tag** column (1–8, 1–24 or A– H), then typing a new position number. The spot order for processing can be changed by click-and-dragging the “circles” beside the **Spot** column to the appropriate position.
- A different spot protocol can be applied to a spot by clicking in the corresponding row of the **Spot Protocol** column, then selecting a new spot protocol from the drop-down list.
- Spectrum comments can be entered for each sample by clicking once on the appropriate **Spectrum Comment** field and typing a comment.
- To run the chip protocol, press the **Start Running** button or select **Execute Chip Protocol** from the **Instrument** menu.
- To insert a line in a chip protocol (i.e., to add a spot), press the <Insert> key, or select **Insert** from the **Edit** menu.
- Spots can be deleted from the run by selecting them and pressing the <Delete> key, or by choosing **Delete** from the **Edit** menu.



- To open an existing chip protocol, press the arrow next to the **Chip Protocol** toolbar button and select the protocol from the drop-down list.

Managing chip protocols

Chip protocols reside in the **Chip** subfolder in the user's folder inside the **Ciphergen ProteinChip Software 3.0** folder (accessible via the **Windows Explorer**, Figure 1-19). Like all other protocols, chip protocols can be shared with other users by copying the protocol into that user's **Chip** subfolder.

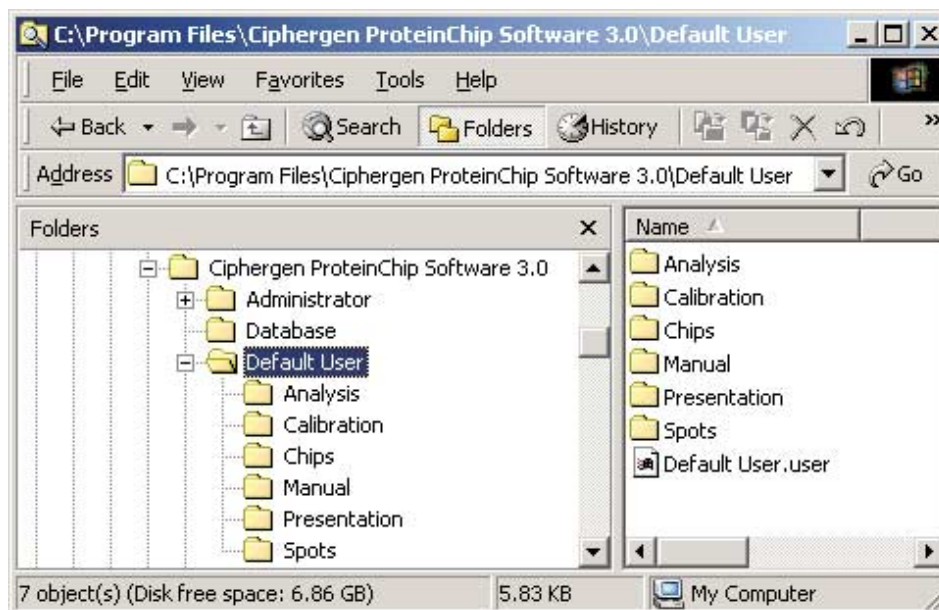


Figure 1-19: *Windows Explorer* view of the contents of the **Ciphergen ProteinChip Software 3.0** folder. The sub-folders within the user's folder are labelled with the type of protocol they contain.

Protocols can be transferred, shared, or copied between users with no restrictions. To delete a protocol, use **Windows Explorer** to remove the protocol from its subfolder.

Labeling peaks



Full Spectrum
button

To view the entire spectrum, press the <Home> key on the keyboard, or click the **Full Spectrum** toolbar button in the **Data Analysis** toolbar.

To label the peaks in a spectrum automatically, select the spectrum, then click the **Identify Peaks** toolbar button (Figure 1-20). The peaks in view in the selected spectrum will be labeled according to the current **Analysis Protocol** settings (see Chapter 4, "Data Analysis" for more on analysis protocols).

To label the peaks in a spectrum manually, click the **Centroid** button in the **Data Analysis** toolbar.

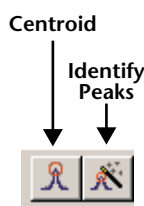


Figure 1-20: The **Centroid** and **Identify Peaks** buttons.

The cursor will change to the centroid cursor (see Figure 1-21). Position the cursor so that the peak to be labeled is centered between the cursor's vertical lines, then click to label the peak.

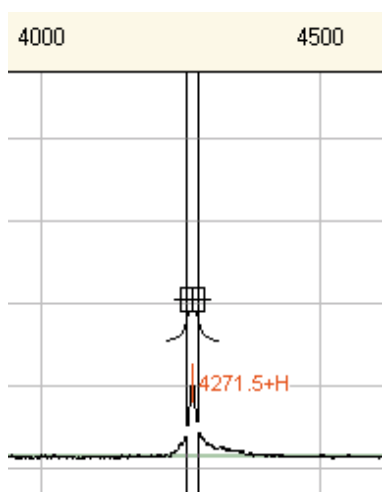


Figure 1-21: Labeling a peak with the centroid cursor.

To remove a single peak label, click the **Centroid** button to switch to the centroid cursor, move the cursor over the peak, then right-click and select **Clear** from the pop-up menu.



To remove all of the peak labels in the current view, click the **Clear Peaks** button in the **Data Analysis** toolbar. Clicking this button clears all peak labels in the currently displayed spectrum window.

Calibration

ProteinChip reader data is a set of time-of-flight measurements, which are converted to mass measurements through the calibration equation. When a spectrum is "calibrated" it is simply assigned a new calibration equation. The underlying data remains unchanged.

To calibrate a spectrum:

1. Open the **Calibration** dialog (Figure 1-22) by clicking the **Calibrate** toolbar button. The cursor will change to the calibration cursor.



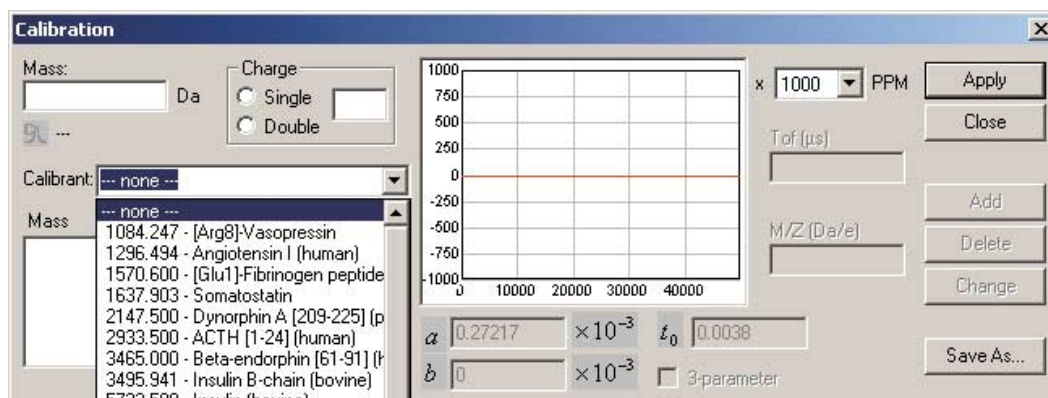


Figure 1-22: The **Calibration** dialog and the calibration cursor.

- Click on a calibrant peak in the spectrum using the calibration cursor. The calculated mass of the peak will appear next to the cursor icon in the **Calibration** dialog (Figure 1-23).
- If the calibration peak is in the **Calibrants** drop-down list in the **Calibration** dialog, select it from the list. Otherwise, enter the correct molecular weight (MW) in the **Mass** field.

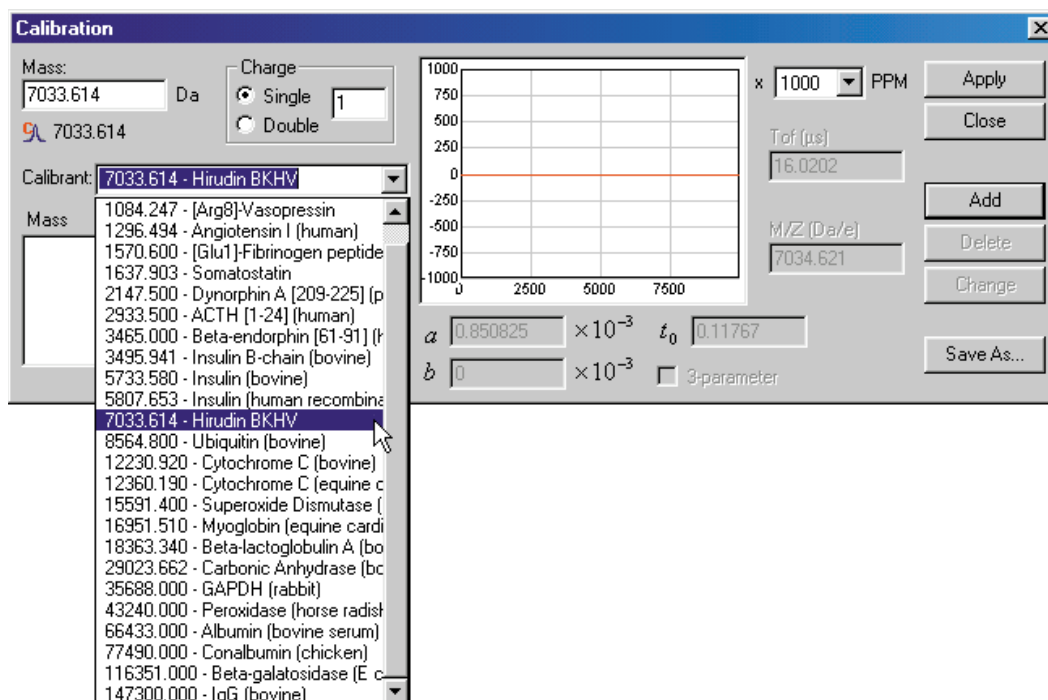


Figure 1-23: Selecting a calibrant.

4. Make sure that the **Charge** value (single or double) is correct.
5. Click the **Add** button and the peak will be added to the calibration for this spectrum.
6. Repeat steps 2 to 5 for any remaining calibration peaks in the spectrum.
7. Review the residual plot (in the box near the center of the **Calibration** dialog, Figure 1-24). It shows the fit of the calibration points to the calculated calibration curve, and is used to determine if any of the mass calibration standards are misidentified or are significantly outside the expected mass accuracy. In a good calibration equation, the calibration points (blue cross-hair boxes) will lie close to the calibration curve in the residual plot (represented by the horizontal red line). If any of the calibration points significantly deviate from the calibration curve, the calibration equation may be inaccurate.

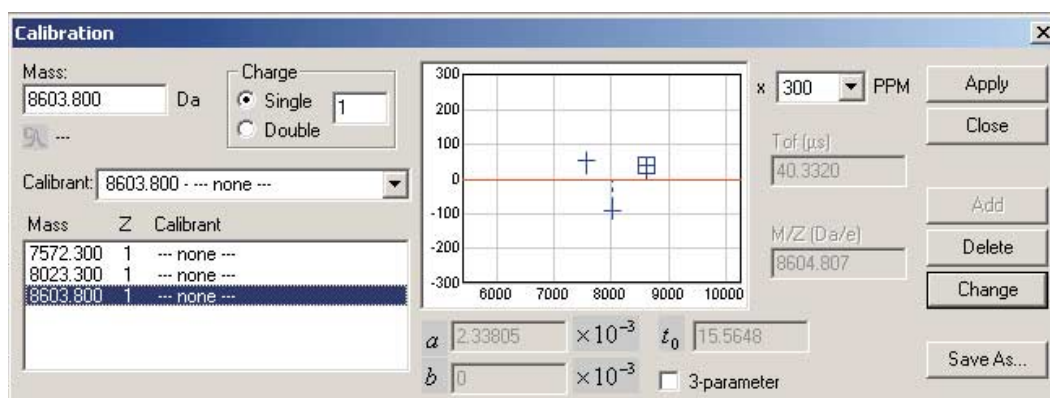


Figure 1-24: The residual plot in the **Calibration** dialog.

8. Individual calibrants can be changed or deleted from the list of calibrants — simply select the calibrant from the list, then click the appropriate button (**Delete** or **Change**).
9. Click the **Apply** button to apply the completed calibration to the spectrum.
10. When finished, click the **Close** button to close the **Calibration** dialog.

Chapter 2: Managing Data and Users

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About managing data

ProteinChip Software version 3.0 stores spectra and experiment files into databases, rather than as files. The major benefit of this approach is that data can be easily rearranged into different experiments for analysis. Data can be located and sorted by key data properties including the date of data acquisition, the ProteinChip Array type, the laser intensity, and additional fields. This makes it easier to construct experiments that contain a consistent group of conditions for expression analysis.

When you create a ProteinChip database, a new folder is created for the database and a file called **ProteinChip.mdb** is placed in the folder. As you add experiments and spectra to the database, additional subfolders are created within the database folder. The folder and file names are created automatically by the software, and are not of use to the software users. The data are accessed through ProteinChip Software, which connects the ProteinChip database file to the various spectra files contained in the folders.



IMPORTANT: Do not alter, delete or rename any of the files in a ProteinChip database folder. Doing so will corrupt the ProteinChip database.

The database connection wizard

The database connection wizard allows you to create a new ProteinChip database or connect to an existing database.

Creating a new database

If you are already logged into the software and would like to create a new database,

1. Select the **New | Database** item from the **File** menu. The **Database Connection Wizard** dialog will open (Figure 2-1).



Figure 2-1: The opening page of the **Database Connection Wizard** dialog.

2. Select **Create a new database** and press the **Next** button. The second page of the wizard will be displayed, which will allow you to select the path for the new database folder. The program will default to the folder in which the ProteinChip software has been installed.



Figure 2-2: The second page of the *Database Connection Wizard* dialog.

3. Press the ... button to browse to other directories using the **Select Folder** dialog (Figure 2-3). When the desired folder is selected, press the **Next** button.

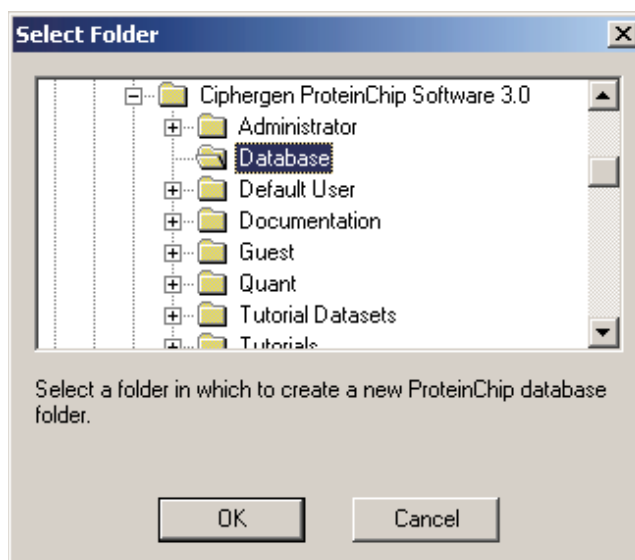


Figure 2-3: The *Select Folder* dialog.

4. Enter the name of the new database folder.



Figure 2-4: Naming the new database folder.

5. A message will appear after pressing the **Finish** button confirming the location of the new database (Figure 2-5).



Figure 2-5: The Confirm alert.

Browsing for an existing database

1. Launch the database connection wizard.



Figure 2-6: Using the Database Connection Wizard dialog to connect to an existing database.

2. Select **Connect to existing database** and click **Next**. The **Select ProteinChip Database Folder** dialog will open.
3. Browse your computer and/or network for the database folder by pressing the ... button to bring up the browse window. When a folder containing a database is highlighted, the creation date of the database and the data of the last access are displayed (Figure 2-7).

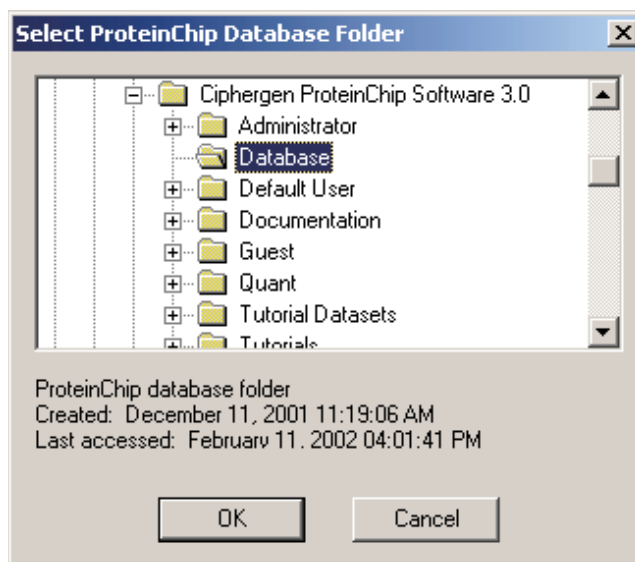
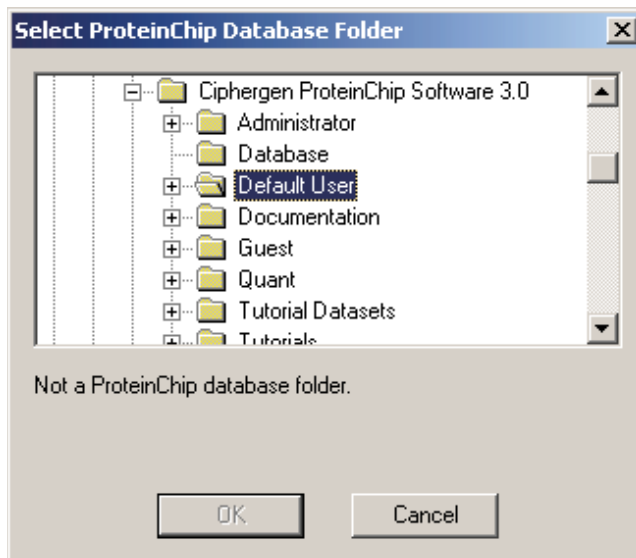


Figure 2-7: The Select Protein Chip Database Folder dialog as it appears when a folder containing a ProteinChip database is highlighted.

If the folder doesn't contain a database, the dialog displays the message **Not a ProteinChip database folder**.



*Figure 2-8: The **Select ProteinChip Database Folder** dialog as it appears when a folder that doesn't contain a ProteinChip database is highlighted.*

4. Once the preferred database folder is found, close the browse window by pressing the **OK** button.
5. Verify the path to the database folder you selected, then press **Finish**. You will then be able to log into the database.

Logging in and out of databases

Each database is created with a default project and two default user names: **Administrator** and **Guest**. Both of these user names are created with blank passwords that can be changed after the initial login.

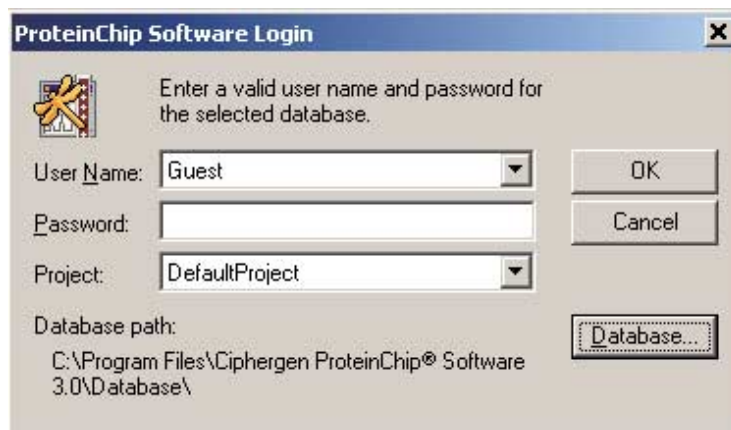


Figure 2-9: The *ProteinChip Software Login* dialog.

Initially, choose **Administrator** from the drop down menu. The path for the active database is displayed at the bottom of the window. Once logged in, passwords and new users can be created from the **User Manager** dialog accessed via the **Options** menu. Only users specifically created for the current database are visible — if a user was created on the same computer but for a different database, their name won't be visible (see “*Managing users*” on page 40 for more information).

Once your session with ProteinChip Software is finished, you can log out of the program by selecting **Log Off** from the **File** menu. Logging off helps prevent other users from accidentally saving data into the wrong database.

Copying or moving a ProteinChip database

ProteinChip databases cannot be moved or copied from within the ProteinChip software. Instead, use **Windows Explorer** to copy or move the entire database folder.



IMPORTANT: Do not alter, delete or rename any of the files in a ProteinChip database folder. Doing so will corrupt the ProteinChip database.

To move or copy a database folder,

1. Exit the ProteinChip Software.
2. Open **Windows Explorer**.
3. Click the file or folder you want to move or copy.
4. On the **Edit** menu, click **Cut** (or **Copy**).
5. Browse to the folder in which you want to put the database folder or file.
6. On the **Edit** menu, click **Paste**.

Data management tutorial

Now that you have created a database, this tutorial will guide you through creating a project, importing multiple experiment files into the project, and saving the experiments.

Creating or opening a project

All data saved into a database resides within a given project. New databases include a project named **DefaultProject**. You can create additional projects, as well as edit the properties of the projects within a database. The name of the current (open) project is shown in the **Status** bar, at the lower right corner of the ProteinChip Software window.

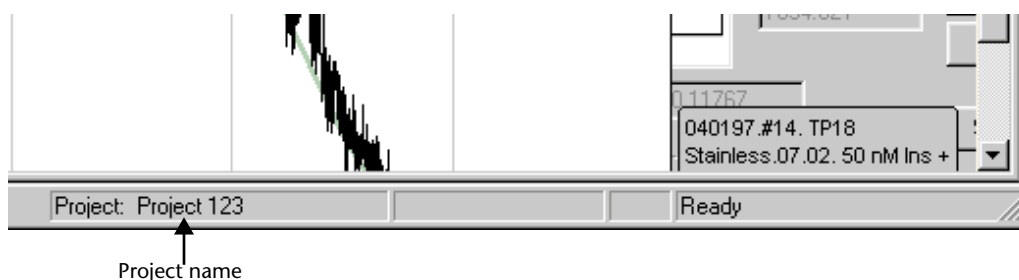


Figure 2-10: The current project name is displayed in the *Status* bar.

1. To create a new project, select the **New | Project...** item from the **File** menu, which will open the **New Project** dialog (Figure 2-11).

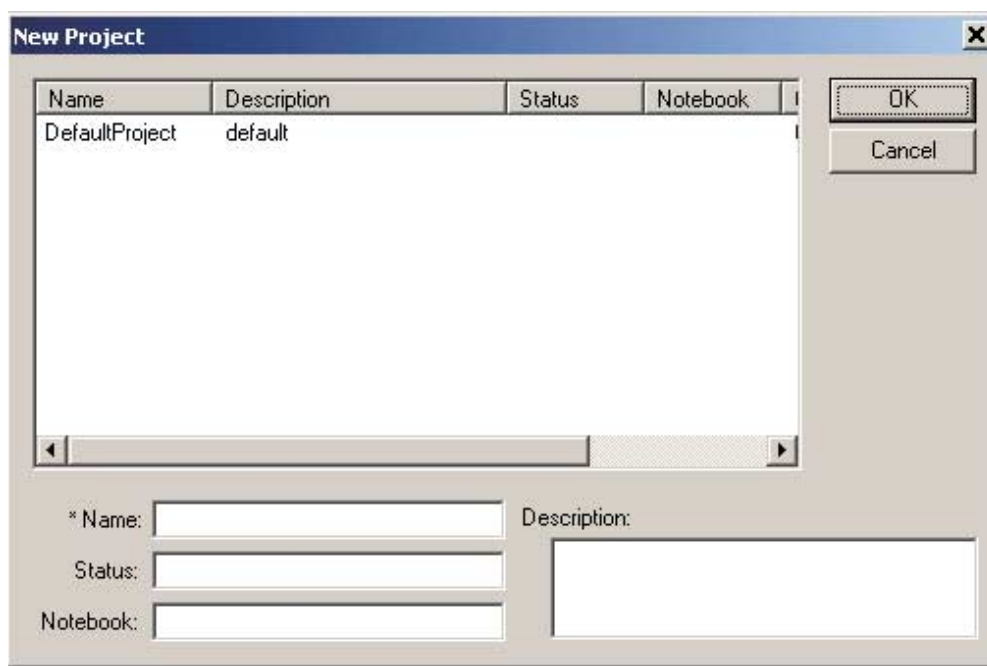


Figure 2-11: The *New Project* dialog.

2. Enter **Import Tutorial** for the project name. You can also assign a description, status, and notebook number to a project. When the information is entered, press **OK**. The new project will be created, and the software will automatically open the new project. The project name is shown at the bottom of the main window in the **Status** bar (refer to Figure A-1 on page 119 for the location of the **Status** bar).
3. To open an existing project, select **Open | Project** from the **File** menu.



NOTE: In version 3 of the ProteinChip Software, it is not possible to delete a project from a database. If the number of projects in a database becomes unmanageable, you may wish to create a new database.

Importing a single experiment file into a project

1. Open the **Import** window by selecting **Import | Experiment** from the **File** menu.
2. Locate the **All-in-1 Protein MW Standards.xpt** experiment file located in the **C:\Program Files\Ciphergen ProteinChip® Software 3.0\Tutorials\Standards** folder and highlight the file. Press **Open**. The file will be shown in the software.
3. To save the file into the database, click the **Save** button on the **Standard** toolbar, or select **Save** from the **File** menu. The **Save Experiment** dialog will open.



Save
button

Save Experiment

Project: Import Tutorial

Name	Description	Created by

Name: All-in-1 Protein MW Standards

Description:

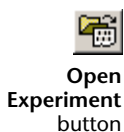
OK Cancel

If you want to save to a file, press cancel and then use File | Export

Figure 2-12: Saving a new experiment into a project.

4. Select the **Import Tutorial** project in the **Project** drop-down list.

5. Enter **All-in-1 Protein MW Standards** as the experiment name.
6. Press **OK**. The file is now saved to the **Import Tutorial** project in your database. All the sample properties associated with the file will be automatically entered into the database as well.



1. To open the file from the database, click the **Open Experiment** button on the **Standard** toolbar, or select **Open | Experiment** from the **File** menu. This will bring up the **Open Experiment** dialog, allowing you to open experiments saved in the current database.

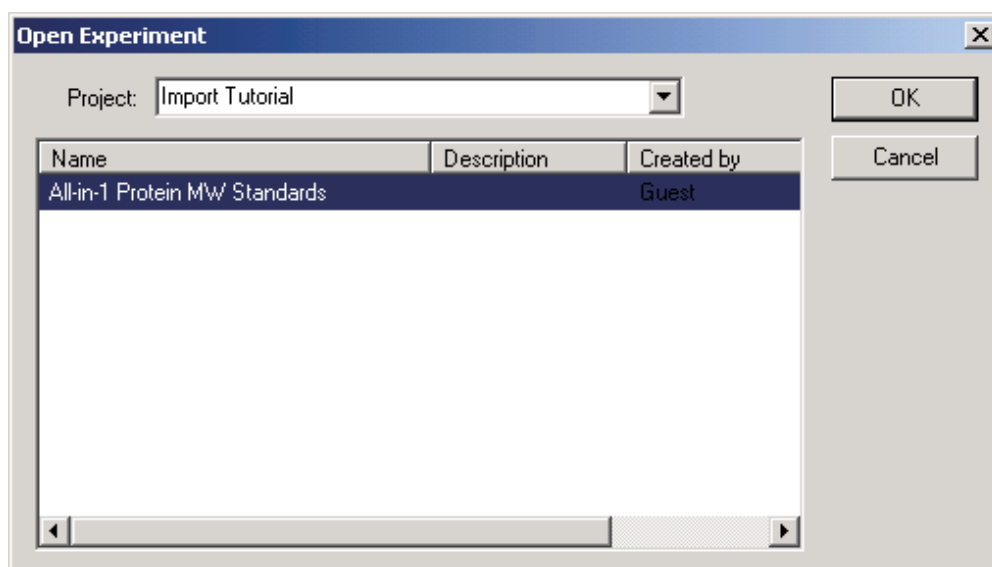


Figure 2-13: Selecting an experiment to open.

2. The **Project** field contains a drop-down list of projects. When a project is selected, all of the experiments in that project will be displayed below the **Project** field. Select the **Import Tutorial** project, then select the **All-in-1 Protein MW Standards** experiment. Click **OK** to return to the main software window.

Batch importing experiment files

The **Open Experiment** dialog only allows you to open experiment files saved in the current database. If you want to use files stored in another database, or perhaps a file sent by a colleague as an .xpt file, you will need to import the experiment files to the current database.

1. Select **Batch...** from the **File | Import** menu to open the **Batch Import and Save to Database** dialog (Figure 2-14).

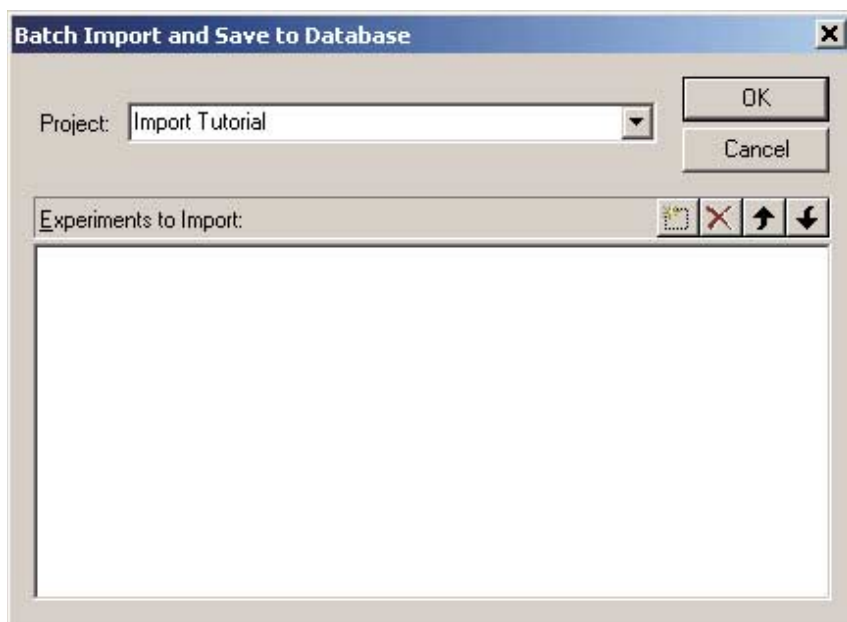


Figure 2-14: The *Batch Import and Save to Database* dialog.

2. Select **Import Tutorial** project from the **Project** dropdown menu.

3. Press the **New (Insert)** button to begin selecting files to import. The ... button will appear below the down arrow button.


New (Insert)
button

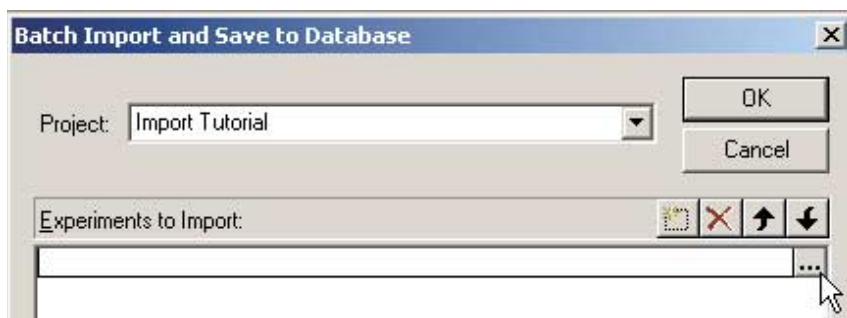


Figure 2-15: Clicking the *New* button in the *Batch Import and Save to Database* dialog causes the... button to appear.

- Click the ... button to open the **Select Experiments to Import** dialog (Figure 2-16).

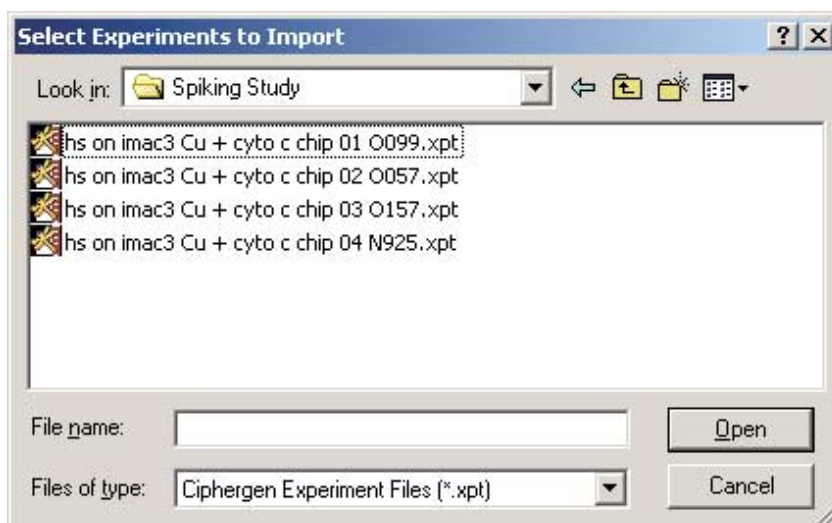


Figure 2-16: The *Select Experiments to Import* dialog.

- Use the dialog to browse into the **C:\Program Files\Ciphergen ProteinChip® Software 3.0\Tutorial Datasets\Spiking Study** folder on your hard drive.
- Highlight all four spectra in the folder by highlighting the first spectrum, then holding down the <Shift> key and highlighting the last spectrum. Press **Open** to load the files into the **Batch Import and Save to Database** dialog.

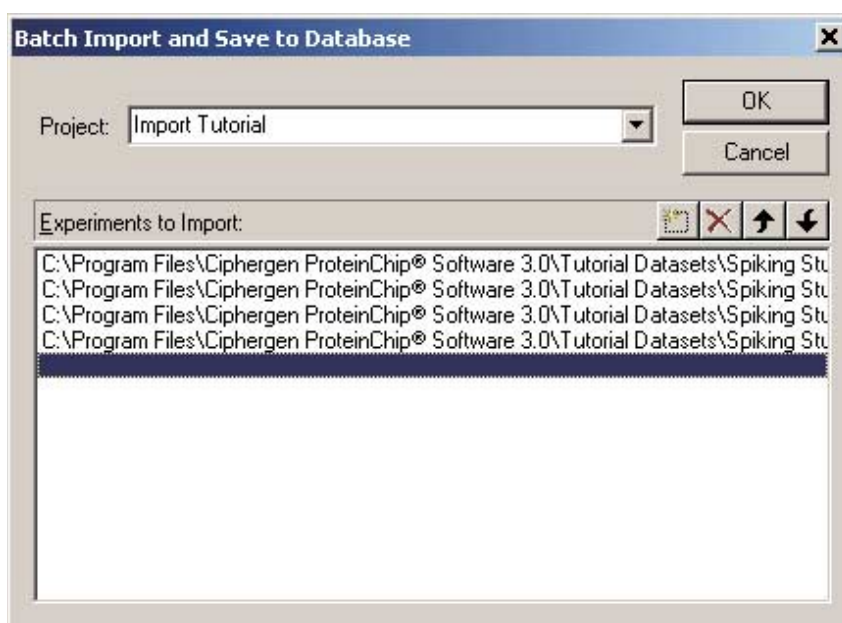


Figure 2-17: The *Batch Import and Save to Database* dialog, with files to be imported.

7. Once the files are loaded, press **OK** to begin the batch import. Each file is loaded into ProteinChip Software 3.0 and then automatically saved into the **Import Tutorial** project in your database.

Exporting experiment files to other databases

Computers that cannot directly connect to your database will not be able to open your files. To exchange files with other ProteinChip Software version 3.0 users, the files must be exported from your database, and then imported onto other computers. Files exported from v.3.0 can not be opened by previous versions such as v.2.1x.

1. To practice exporting an experiment, open the **All-in-1 Protein MW Standard** experiment by choosing **Open | Experiment** from the **File** menu, then selecting the **All-in-1 Protein MW Standard** experiment from the **Import Tutorial** project.
2. Select **Export | Experiment** from the **File** menu. The **Export As** dialog will open.

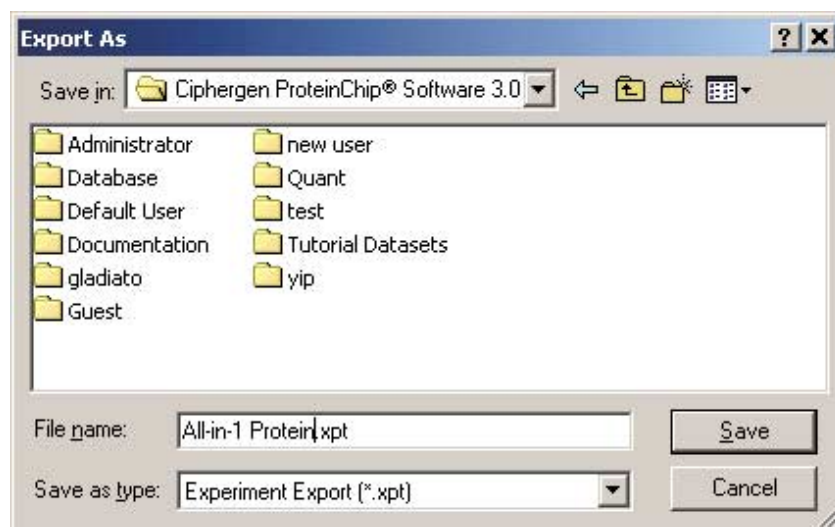


Figure 2-18: The Export As dialog.

3. Select a folder destination, such as **C:\Program Files\Ciphergen ProteinChip® Software 3.0**.
4. Enter the file name **All-in-1 Protein.xpt** and press **Save**. The experiment will be saved as a separate .xpt file that can be transported to another user via email, FTP, or disk.

Managing users

Each new database is automatically created with two users: **Administrator**, and **Guest**. Each database has its own set of users. Users from different databases may have the same name, but each database stores its own private list of passwords, so a particular user's password in one database may not work for the same user name in another database.

New users can be created, and existing users' information and passwords can be modified by logging in as the administrator and making the appropriate changes.

Creating new users



1. From the **Options** menu, select **Manage Users**, or click the **Manage Users** button on the **Data Analysis** toolbar, to open the **User Manager** dialog.

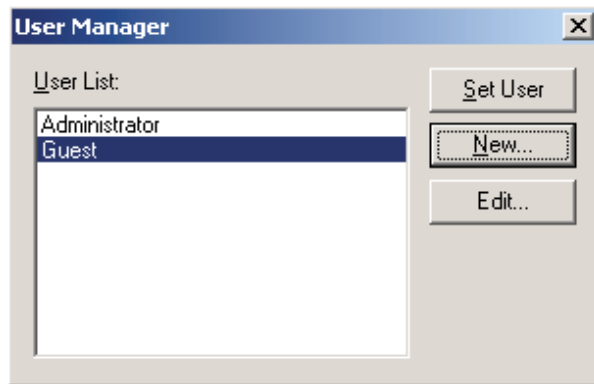


Figure 2-19: The *User Manager* dialog.

2. Click the **New...** button to add a new user. If you are not already logged in as the administrator, The **Log on** dialog will appear, prompting you to do so. Only the administrator account is authorized to add or change users.

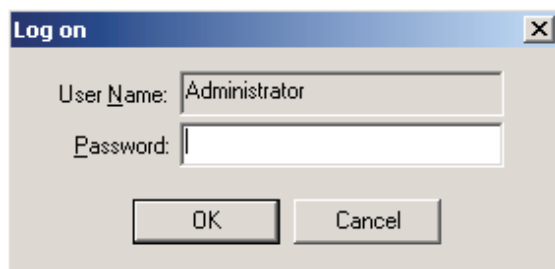


Figure 2-20: The *Log on* dialog.

3. After you have logged in as the administrator, the **Creating New User** dialog will open (Figure 2-21).

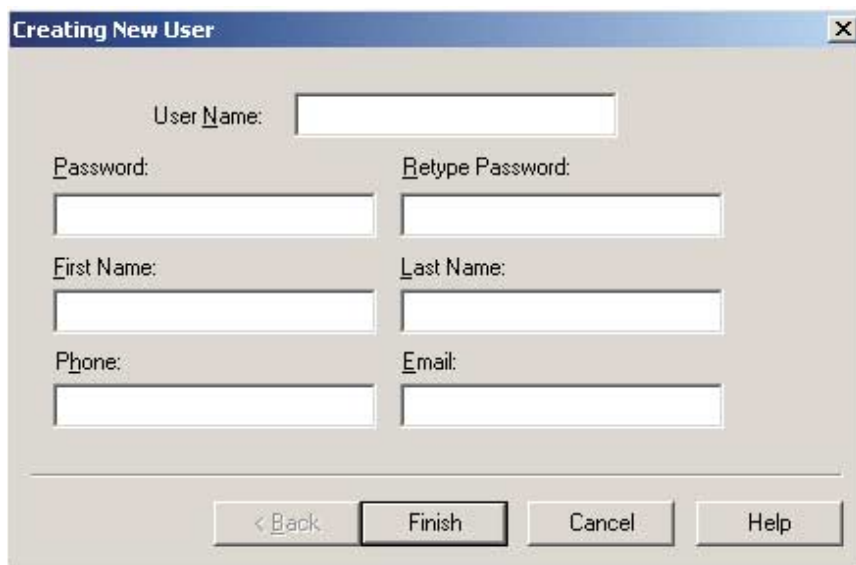
A screenshot of the 'Creating New User' dialog box. It has a title bar with the text 'Creating New User' and a close button (X). The dialog contains several text input fields: 'User Name:', 'Password:', 'Retype Password:', 'First Name:', 'Last Name:', 'Phone:', and 'Email:'. At the bottom, there are four buttons: '< Back', 'Finish', 'Cancel', and 'Help'.

Figure 2-21: The Creating New User dialog.

4. Enter the name and password for the new user. You may also enter additional information about the user. When finished, click **Finish**.



***NOTE:** If the passwords in the **Password** and **Retype Password** fields do not match, you will be prompted to enter them again.*

Modifying users

The administrator can edit any user information, including the password, at any time.

1. In the **User Manager** dialog, select the user to be modified and click **Edit...**

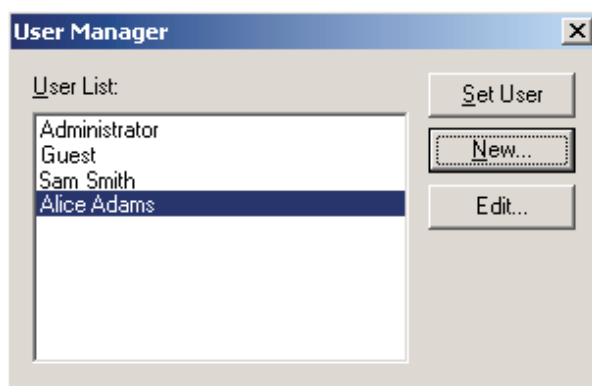
A screenshot of the 'User Manager' dialog box. It has a title bar with the text 'User Manager' and a close button (X). On the left, there is a list box labeled 'User List:' containing the names 'Administrator', 'Guest', 'Sam Smith', and 'Alice Adams'. 'Alice Adams' is currently selected. To the right of the list box are three buttons: 'Set User', 'New...' (with a dotted border), and 'Edit...'.

Figure 2-22: Selecting a user to modify.

2. The **Edit User** dialog will open. The name of the selected user will be shown in gray at the top of the dialog.

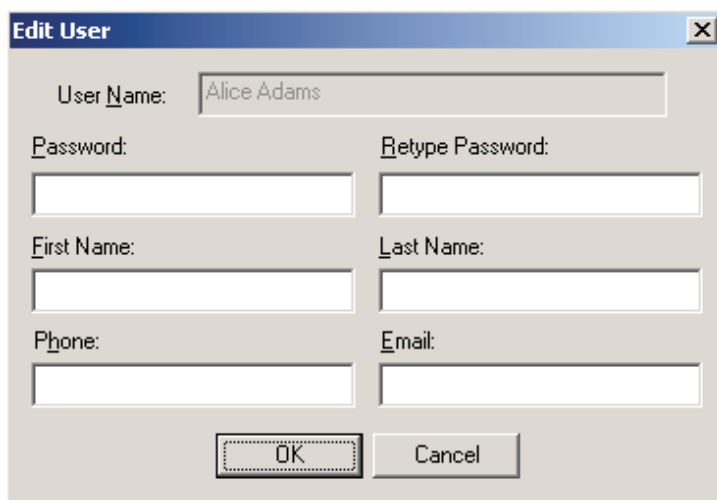


Figure 2-23: The Edit User dialog.

3. Make any changes, then click **OK** to accept the changes, or **Cancel** to leave the user unchanged.



NOTE: If a user's password has been forgotten, use the **Edit User** dialog to create a new password for the user.

Deleting users

In ProteinChip Software 3.0, it is not possible to delete a user from the database. To prevent a particular user from accessing the database, use the administrator account to change the user's password.

User-specific files

Some data associated with the instrument and data acquisition is stored in folders within the program installation directory. For each user, a folder is created with sub-folders that contain:

- Analysis protocols
- Calibration protocols
- Calibration equations
- Chip protocols
- Presentation protocols
- Spot protocols

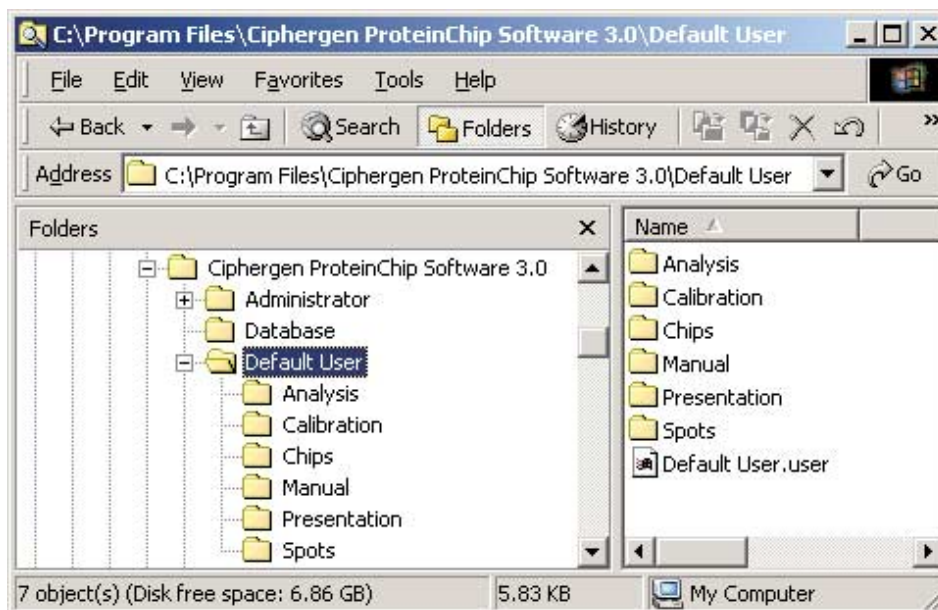


Figure 2-24: *Windows Explorer* view of the contents of the **Ciphergen ProteinChip Software 3.0** folder. The sub-folders within the user's folder are labelled with the type of protocol they contain.

Note that user-specific files are not contained within the ProteinChip database, but are maintained separately.

Chapter 3: Calibration

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About calibration

Calibration specifies how time of flight data is converted to a mass measurement. ProteinChip Software uses two types of calibration:

External Calibration: uses the calibration from one sample to determine the masses in another. You can expect a mass accuracy of 0.5% or better with external calibration when using the model PBS I, and 0.1% or better with the model PBS II reader.

Internal Calibration: used when the sample contains one or more known masses that can be used to calibrate. Mass accuracy is generally 0.05% or better using internal calibration when using the model PBS I, and 0.02% or better when using the model PBS II reader.

Three-parameter calibration

The default calibration model is linear with regards to the relationship between the square of the time of flight and mass-to-charge ratio. A higher-order quadratic calibration allows for an additional degree of freedom, and can be used to generate a more accurate mass value over a large mass range. If you wish to use the 3-parameter fit, make sure that you select at least 4 or 5 calibration points for use in your calibration spectrum.

The Calibration dialog



Calibration settings are adjusted in the **Calibration** dialog (Figure 3-1), which allows selection and centroiding of peaks, and assigning the correct mass and charge to the peaks. The dialog is accessed by clicking the **Calibrate** toolbar button in the **Data Analysis** toolbar.

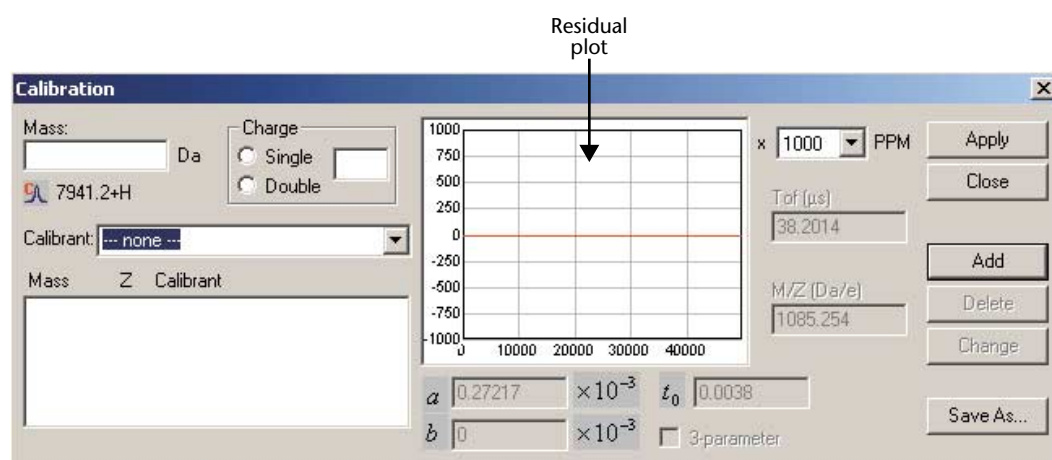


Figure 3-1: The Calibration dialog.

- **Mass:** used to enter the correct mass of the peak being calibrated.
- **Calibrant:** this drop-down list contains the list of defined calibrants. To edit the list of calibrants, select **Calibrants...** from the **Calibration** menu.
- **Charge:** sets the charge of the selected peak.

- **3-Parameter:** Enabling this checkbox allows the software to create a quadratic curve fit. With the checkbox disabled, a linear curve fit is used.
- **Residual Plot:** displays the residual error of the calibration. Since the calibration is calculating the C0 and C1 terms, three or more peaks must be used in a calibration before a residual is possible. The scale of the plot can be adjusted using the combo-box control. The plot is useful for spotting outlying calibration points that may have the wrong mass assigned or might need to be excluded from the calibration to improve accuracy.
- **Add:** adds the currently selected peak to the calibration.
- **Delete:** removes the currently selected calibration point.
- **Change:** adjusts the mass of the currently selected calibration point.

Calibration frequency

The instrument should be calibrated on a regular basis, preferably once per session. Large changes in calibration values from day to day indicate problems with the instrument or with the standards used. If the experiment requires mass accuracy of 0.5% or better, then an external calibration standard should be included on each ProteinChip Array. If mass accuracy of 0.1% or better is needed, an internal calibrant is required.

Viewing the calibration

When the spectrum in the **Data Average** window is calibrated, the calibration equation used for subsequent data acquisition is automatically updated to the new settings. This calibration equation is called the **Default Calibration**, and can be viewed from the **Calibration** menu.

Internally calibrating a spectrum



Calibrate button



Centroid button

1. Open the **Calibration** dialog (refer to Figure 3-1) by clicking the **Calibrate** toolbar button in the **Data Analysis** toolbar.
2. Click the **Centroid** button in the **Data Analysis** toolbar to switch to the centroid cursor, then click on the calibration peak in the spectrum. The current mass of the peak will appear in the **Mass** field.
3. If the calibration peak is in the calibrant list, then select it from the **Calibrant** drop-down. Otherwise, enter its mass in the **Mass** field.
4. Make sure the **Charge** value (**Single** or **Double**) is correct.
5. Click the **Add** button and the peak will be added to the calibration for this spectrum.
6. Repeat steps 2–5 for any remaining calibration peaks in the spectrum.
7. Click **Apply** to apply the calibration to the spectrum.



***TIP:** For increased mass accuracy; select the masses of the calibration standards to span the range of proteins of interest.*

Creating a calibration protocol (tutorial)

Calibration protocols allow creation of a list of calibrants for repeated application as an internal spectrum calibration. This eliminates the need to recalibrate spectra used for calibration. Calibration protocols are especially useful when used in conjunction with the All-in-1 Standards product line. You can also create calibration protocols for your own MW standards.

This tutorial will guide you through the creation of a calibration protocol that can be used with the All-in-1 Peptide Standards.

1. Open the **Calibration Protocols** dialog by selecting **Calibration | Calibration Protocols**.

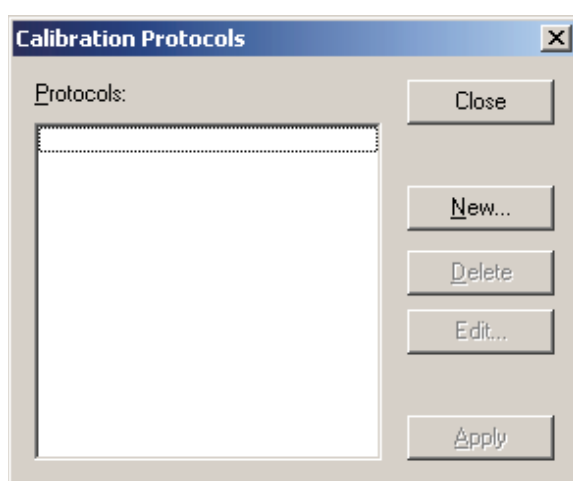


Figure 3-2: The *Calibration Protocols* dialog.

2. Click the **New** button. The **Protocol Name** dialog will open (Figure 3-3).

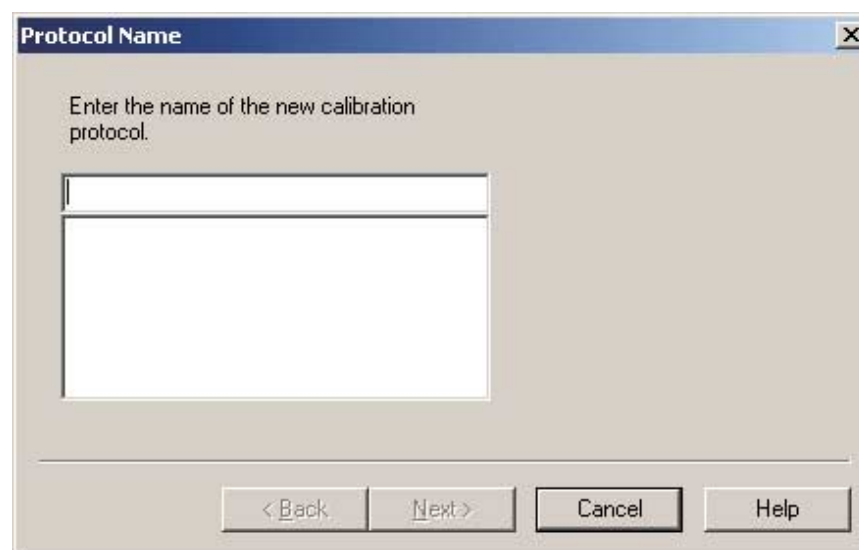


Figure 3-3: The *Protocol Name* dialog.

3. Enter **Calibration Tutorial** as the name for the new calibration protocol, then click **Next**. The **Calibrants** dialog will open.

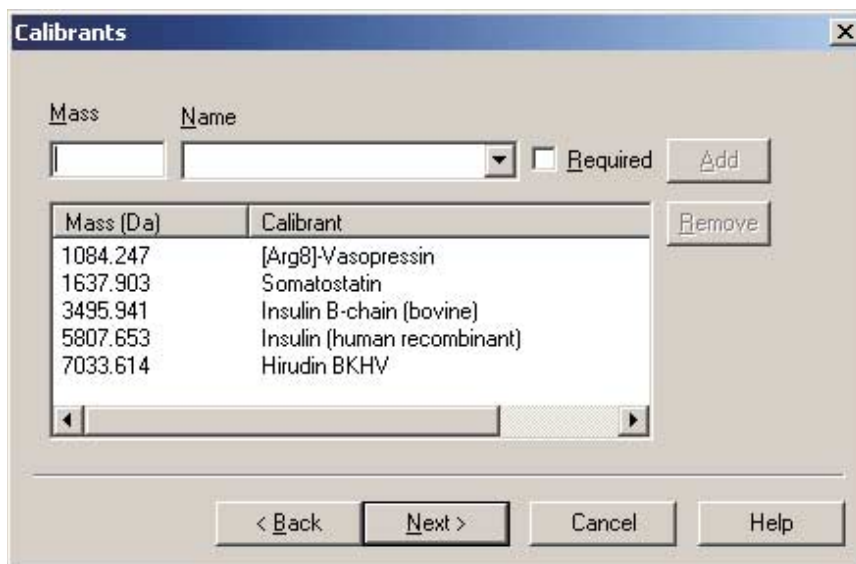


Figure 3-4: The *Calibrants* dialog.

4. Click the **Name** drop-down box to open the list of calibrants, and select **[Arg8]-Vasopressin** (Figure 3-5). The calibrants are listed by size. Click **Add** to include it in the calibration protocol.

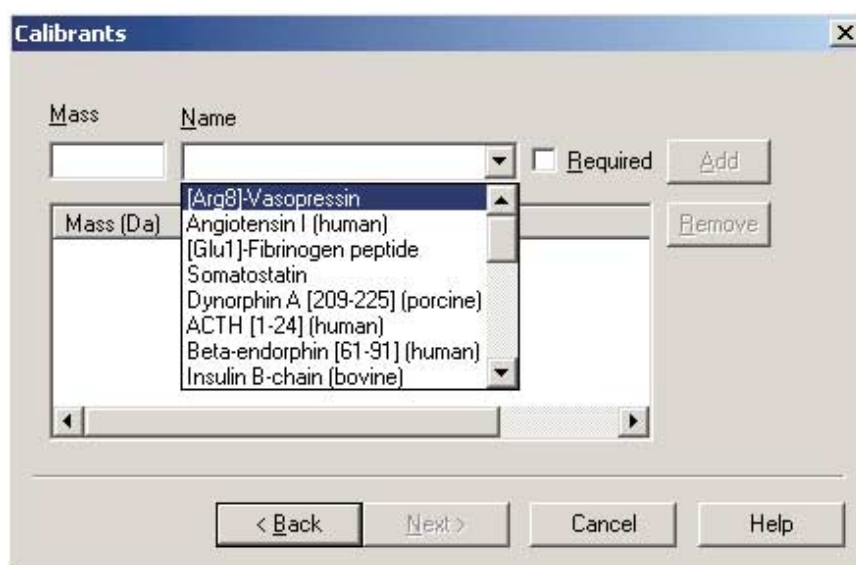


Figure 3-5: Selecting a calibrant to add it to the calibration protocol.

- Repeat this process to add each of the following calibrants: Somatostatin, Insulin B-chain (bovine), Insulin (human recombinant), and Hirudin BKHV. The selected calibrants will be displayed in the scrolling field in the middle of the **Calibrants** dialog. If you include a calibrant by mistake, remove it by selecting it and clicking the **Remove** button (Figure 3-6).

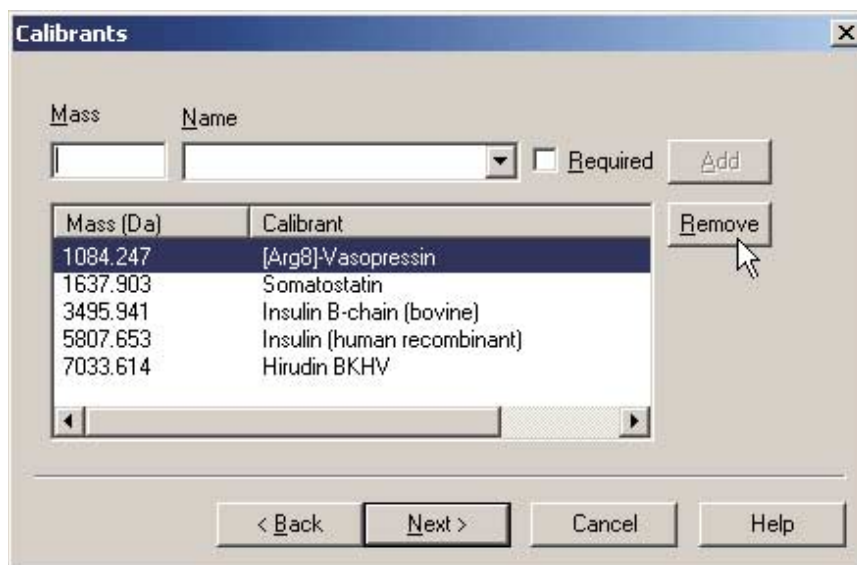


Figure 3-6: Removing a calibrant from the calibration protocol.



NOTE: The order of the calibrants in the list you create is not important.

- When you have finished adding calibrants, click the **Next** button to go to the **Settings** dialog (Figure 3-7). Make note of the settings used to identify the calibrants. The settings can be modified if you have difficulty detecting the calibrants.

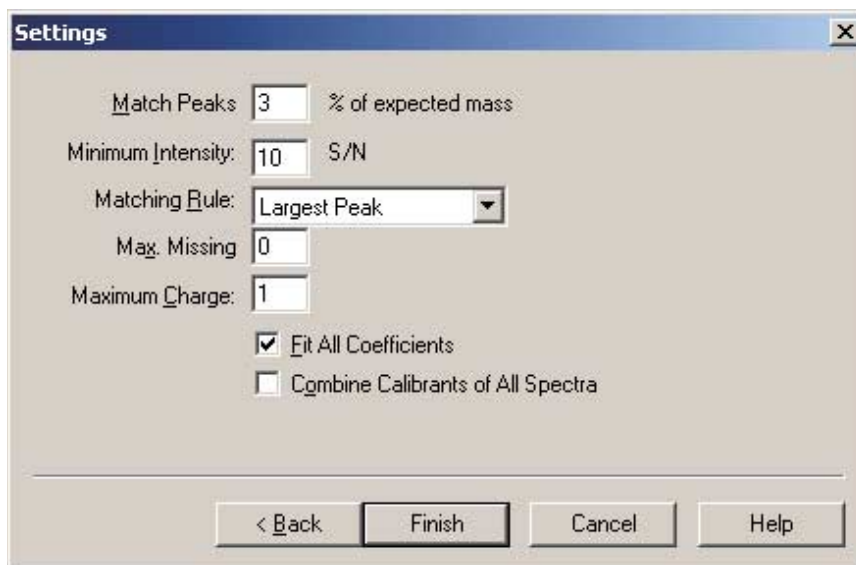


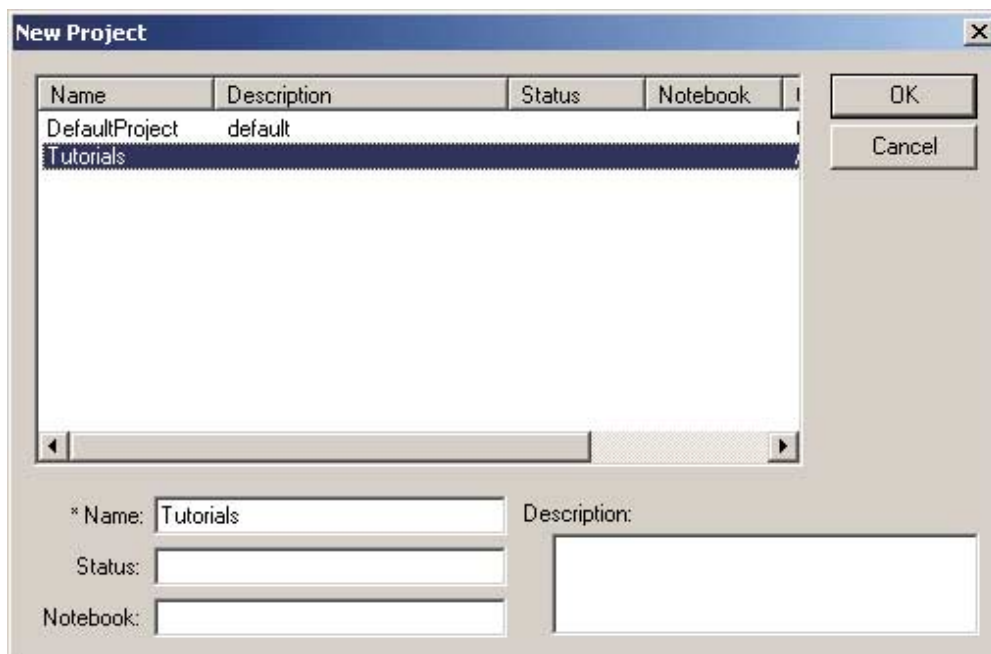
Figure 3-7: The **Settings** dialog.

7. Click **Finish**, which will close the **Calibration Protocols** dialog and save the calibration protocol. The new calibration protocol will be accessible via the **Protocols** list in the **Calibration Protocols** dialog.



NOTE: Calibration protocols are stored in the **Calibration** folder within a database user's folder. Protocols can be shared with other users by using **Windows Explorer** to place a copy of the calibration protocol in the other user's **Calibration** folder. See "User-specific files" on page 43 for more information.

8. Create a new project called **Tutorials** by selecting **File | New | Project** to open the **New Project** dialog (Figure 3-8). Enter **Tutorials** in the name field, then click OK.



*Figure 3-8: Creating a new project called **Tutorials**.*

9. The new **Tutorials** project automatically becomes the current project.
10. Select **File | Import | Experiment**, then import the experiment file **all-in-1 peptide.xpt** from the **C:\Program Files\Ciphergen ProteinChip Software 3.0\Tutorial Datasets\Standards** folder.
11. Choose **Save** from the **File** menu. The **Save Experiment** dialog will open. Make sure **Tutorials** is selected in the **Project** drop-down box, then click **OK** to save **all-in-1 peptide.xpt** to the database in the **Tutorials** project (Figure 3-9).

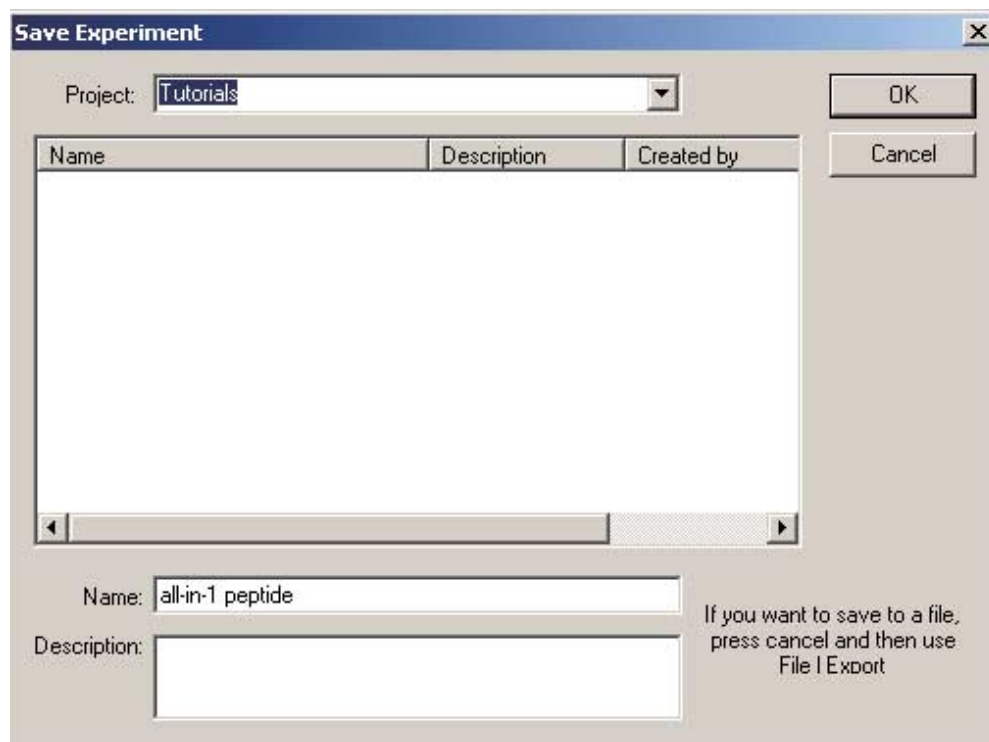


Figure 3-9: Saving the *all-in-1-peptide* experiment in the *Tutorials* project.

12. Select the first spectrum in the experiment by clicking on its sample tag.
13. Apply the **Calibration Tutorial** protocol by selecting **Calibration Tutorial** under the **Calibration | Calibration Protocols** menu. The software will automatically detect the specified calibrants, and generate a new calibration equation for the spectrum.

Managing calibration protocols

Calibration protocols reside in the **Calibration** subfolder in the user's folder inside the **Ciphergen ProteinChip Software 3.0** folder (accessible via the **Windows Explorer**, Figure 3-10). Like all other protocols, calibration protocols can be shared with other users by copying the protocol into that user's **Calibration** subfolder.

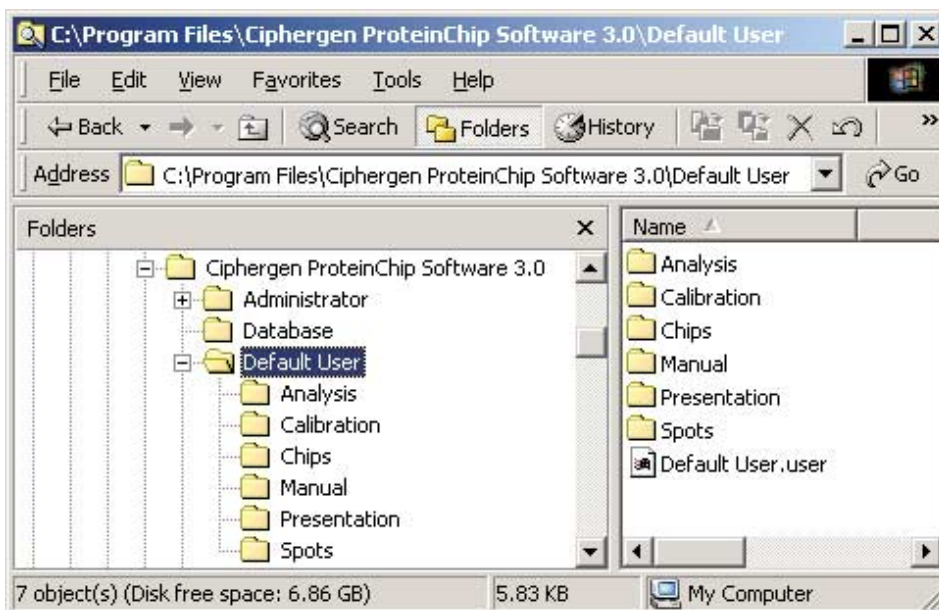


Figure 3-10: *Windows Explorer* view of the contents of the **Ciphergen ProteinChip Software 3.0** folder. The sub-folders within the user's folder are labelled with the type of protocol they contain.

Protocols can be transferred, shared, or copied between users with no restrictions. To delete a protocol, use **Windows Explorer** to remove the protocol from its subfolder.

For further information on calibration protocols, consult the online help file located under the **Help** menu.

Chapter 4: Data Analysis

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About data analysis

ProteinChip Software includes a complete set of analytical tools to extract and process information from raw spectrum data. The software tools have been tuned and should not require any manual adjustment in most cases. However, some basic understanding of how these tools work may be helpful in troubleshooting problems.

The peak detection algorithm

The peak detection algorithm identifies a possible peak when it detects a rise and fall greater than a specified threshold. It then refines the selection by analyzing peak height (default), width, area, or a combination of these properties.

Peak detection thresholds are usually specified in signal-to-noise ratios (S/N) to allow for a uniform criterion across the M/Z axis, because peaks gradually become flatter and more spread out at higher mass. (See *"The noise algorithm"* on page 61, for detailed information.)

The baseline algorithm

The baseline algorithm tries to find a smooth line that follows the trace more or less in the middle of the high frequency noise without rising into the peaks. Its primary purpose is to correct for artifacts from the EAM (Energy Absorbing Molecules) on the spectra although the algorithm is flexible enough to flatten the spectrum trace in most cases regardless of the cause. However, it is occasionally necessary to manually adjust the algorithm parameters to get the algorithm to work. The following is an example in which the baseline algorithm fails to track the spectrum.

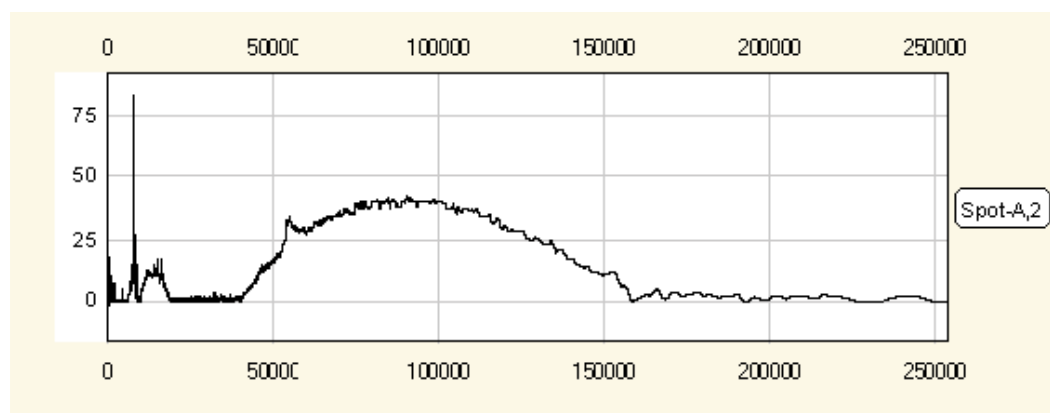


Figure 4-1: Sample data with baseline correction applied to it. The large “hump” in the data indicate that the baseline algorithm is not smoothing the data enough.

In order to see what the algorithm does, turn off baseline correction and also turn on the baseline display (enable **Baseline** on the **Display** page of the **Presentation Protocol Properties** dialog. See *"The Display page"* on page 106 for more information).

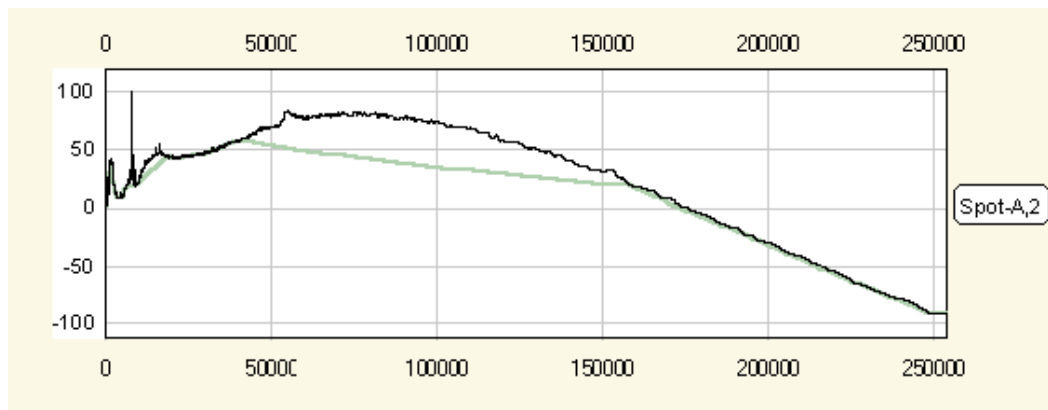


Figure 4-2: Sample data with baseline correction turned off, and the baseline display turned on (green line).

The large gap between the spectrum and the baseline indicate the way in which the algorithm failed and produced the spectrum in Figure 4-1. To correct the problem in this case, the baseline **Fitting width** is reduced in the **Baseline** page of the **Analysis Protocol Properties** dialog until the baseline closely tracks the spectrum along its entire length.

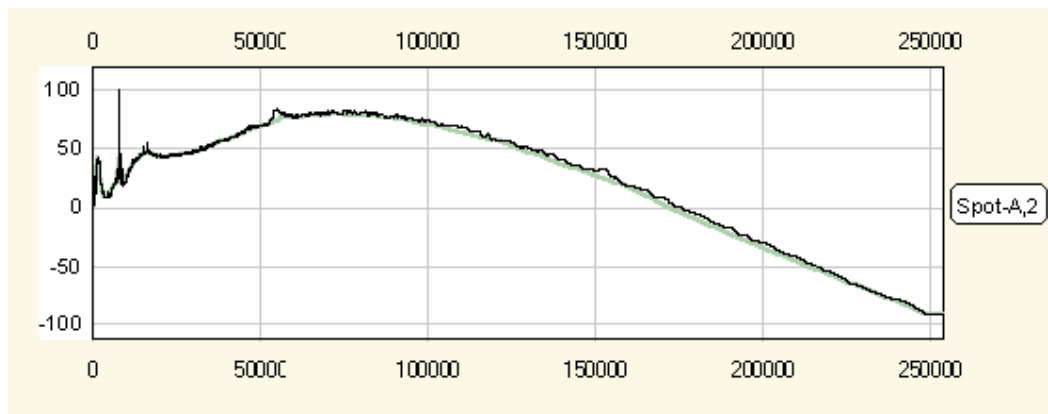


Figure 4-3: Sample data with baseline correction turned off, and baseline display turned on (green line). The **Fitting width** has been reduced until the baseline closely follows the spectrum.

Re-enabling baseline correction should yield the expected result — a relatively flat, smooth spectrum with sharply distinguished peaks (Figure 4-4).

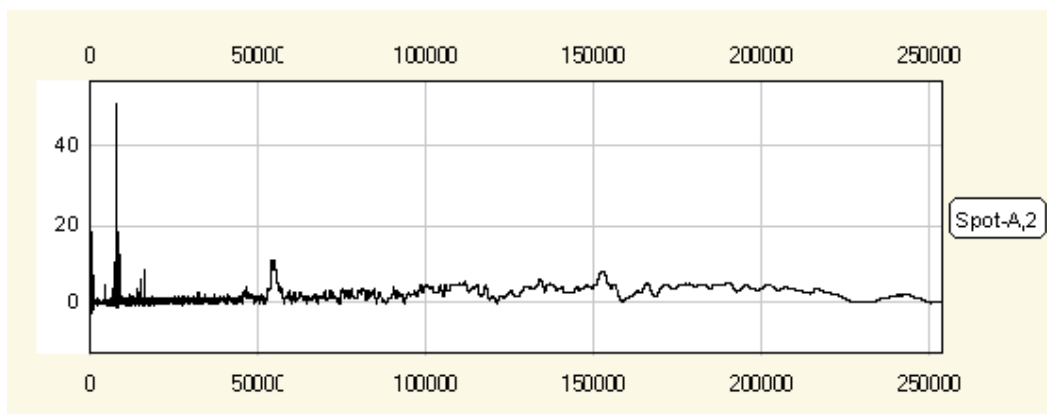


Figure 4-4: Sample data with baseline correction, after baseline correction has been adjusted.

The filtering algorithm

The default **Average** filter should suffice in most cases but the **Savitsky-Golay** filter is more suitable for peptide ID applications.

The peak area algorithm

The peak detection algorithm can optionally cull peaks by peak area. This requires knowledge of the peak boundaries in addition to peak height. There are currently three options for defining peak boundaries in the software. (These options are described in “*The Area page*” on page 67.)

Occasionally the peak area algorithm fails to determine or over-estimates the area of shoulder peaks. The **Show Peak Boundaries** option can be used to troubleshoot such problems. Nevertheless, peak area can be a useful culling criterion for high mass peaks, which tend to be flat and spread out.

The noise algorithm

“Noise” is a measure of the variation in the signal, not of the signal itself. To visualize how much noise the software calculates to be present in a spectrum, activate the **Noise** checkbox on the **Display** page of the **Presentation Protocol Properties** dialog.

If the data are being filtered, the noise may appear to be quite low. This is due to the way noise is calculated. Since the noise varies along the M/Z axis, the noise algorithm divides the axis into a pre-defined number of equal segments when estimating the noise. The noise at a location x_0 is estimated using the standard error of a linear regression of the trace data points in a segment surrounding x_0 . However, the presence of peaks in a spectrum distorts this computation. The algorithm compensates for the distortion by heuristically using only a fraction of the trace’s data points to estimate the noise. It also smooths the resulting noise function rather heavily.

Analysis protocols



Analysis protocols group together parameters used for calculations performed on a spectrum. Analysis protocols are created and edited in the **Analysis Protocol Properties** dialog (refer to Figure 4-5), accessed by selecting **Analysis Protocol Properties...** from the **Options** menu or by clicking the **Analysis Protocol** button in the **Data Analysis** toolbar.

The Analysis Protocol Properties dialog

The Protocols page

The **Protocols** page of the **Analysis Protocol Properties** dialog (Figure 4-5) allows previously saved analysis protocols to be applied to the current spectra, saving the current analysis protocol for later use in another spectrum, deleting protocols, and resetting a protocol to factory defaults.

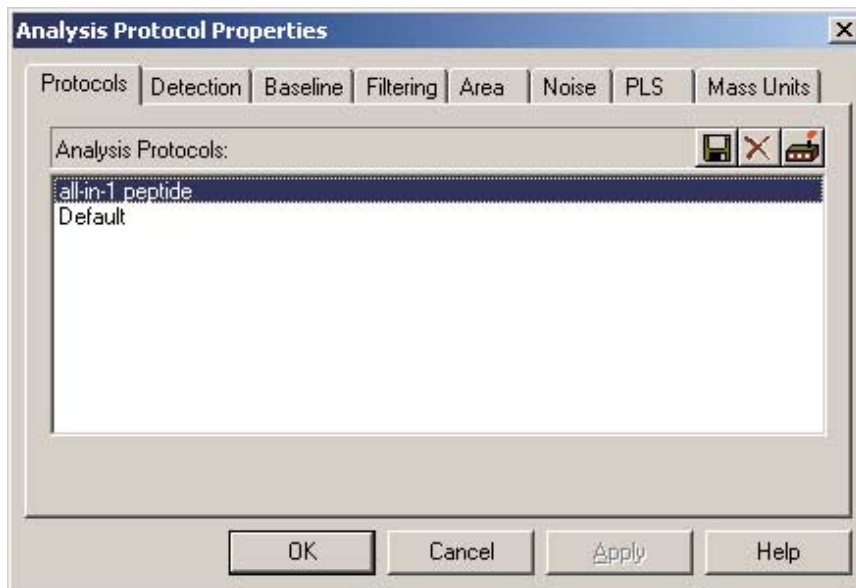


Figure 4-5: The **Protocols** page of the **Analysis Protocol Properties** dialog.



TIP: Analysis and presentation protocols can be applied to multiple spectra in experiment mode. Hold down the <Shift> key (for contiguous selection) or <Ctrl> key (for discontinuous selection) and select the spectra tags, or press Ctrl-A to select all of the spectra in an experiment, then open the **Analysis Protocol Properties** dialog.

- **Save As** (small disk picture): opens a dialog that allows you to re-name and save the protocol selected in the **Analysis Protocols** list.
- **Delete** (X): deletes the protocol selected in the **Analysis Protocols** list.
- **Factory Defaults** (small picture of a factory): resets the selected protocol to the default settings.

The Detection page

The **Detection** page of the **Analysis Protocol Properties** dialog (Figure 4-6) controls the parameters for automatic and manual peak centroiding.

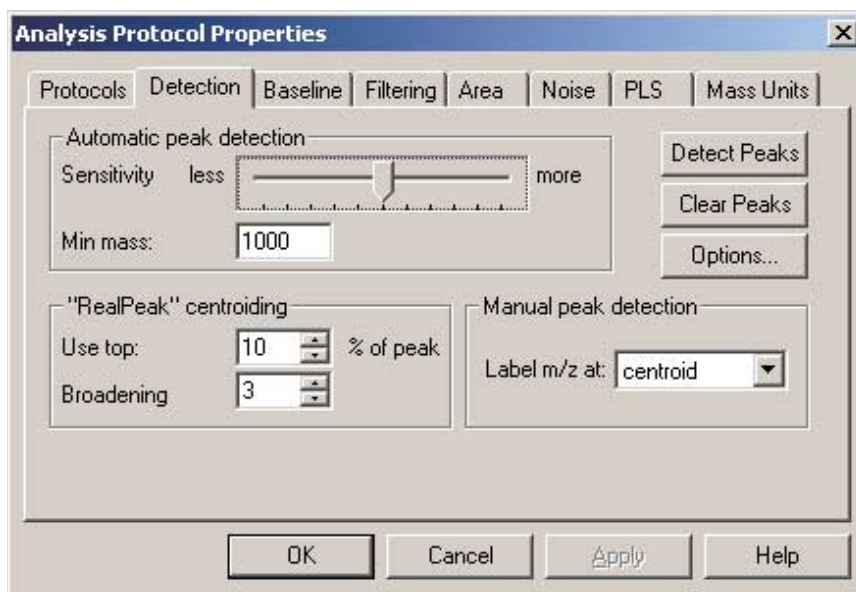


Figure 4-6: The **Detection** page of the **Analysis Protocol Properties** dialog.

- **Automatic peak detection:** this slider varies the peak detection sensitivity. The actual numerical values that the slider changes are set in the **Automatic Peak Detection Options** dialog, described in the next section.
- **Min mass:** sets the minimum mass that the automatic peak detection will use in attempting to find a peak.
- **Detect Peaks** and **Clear Peaks:** buttons that allow experimentation with new peak detection settings on the currently selected spectrum.
- **Options...:** opens the **Automatic Peak Detection** dialog, described below.
- **"RealPeak" centroiding:** the options in this section of the dialog define the region to be used for assigning the centroid.
- **Use top:** specifies the percentage of the peak to use in centroiding.
- **Broadening:** the value used to adapt the centroid cursor for wider values at higher masses.
- **Manual peak detection:** this setting allows you to choose between manually labeling peaks either at the true centroid or at the location of the mouse click.

Automatic Peak Detection Options

The **Automatic Peak Detection** dialog (Figure 4-7) controls how peaks are found. The **Sensitivity** slider on the **Detection** page of the **Analysis Protocol Properties** dialog interpolates between the low and high sensitivity values entered in this dialog.

	Low Sensitivity	High Sensitivity	
Minimum valley depth	10	2	times the noise
Peak conditions			
<input checked="" type="checkbox"/> Height	10	2	times the noise
<input type="checkbox"/> Width at half height	0.5	0.05	times expected peak width
<input type="checkbox"/> Area	1	1	

Figure 4-7: *The Automatic Peak Detection dialog.*

- **Minimum valley depth:** controls the minimum difference in height between a peak and its nearest neighbor.
- **Peak conditions:** these checkboxes can be marked to limit the peaks found by several different criteria:
- **Height:** controls the minimum height from baseline that will be considered a peak. Peaks below this value will be discarded.
- **Width at half height:** controls the minimum width that will be acceptable for a peak.
- **Area:** rejects peaks with areas lower than the specified value.

The Baseline page



Figure 4-8: The **Baseline** page of the **Analysis Protocol Properties** dialog.



TIP: The **Apply** button can be used to see the effect of changing a parameter before closing the dialog — handy for changing the baseline or filtering settings and immediately observing the changes on the spectrum.

- **Smooth before fitting baseline:** using this option can improve the accuracy of the baseline by fitting the data to a moving average before calculating the baseline.
- **Window:** specifies the width of the moving average filter used.
- **Automatic:** selecting the **Automatic** checkbox assigns the automatic fitting width, which works well for most data. Deselecting the **Automatic** checkbox enables the **Fitting width** field, allowing you to manually select a fitting width for the baseline segments.

The Filtering page

Filtering can significantly improve signal-to-noise ratios by removing high frequency noise from the spectrum. However, excessive filter widths can distort peaks and reduce resolution.

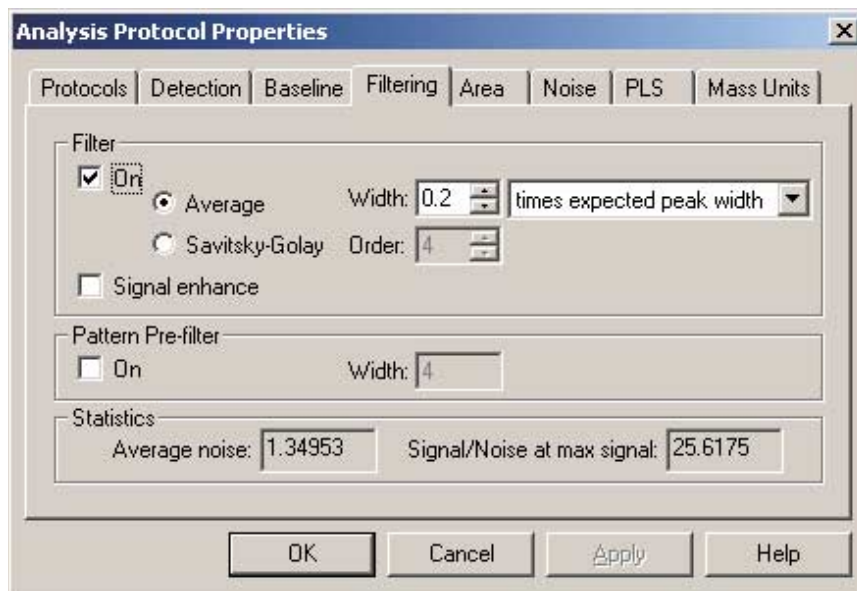


Figure 4-9: The **Filtering** page of the **Analysis Protocol Properties** dialog.

- **Average:** if selected, a moving average filter will be calculated. The units of the filter width can be **times expected peak width**, **Constant Daltons** or **Constant, Points**. Using **times expected peak width** makes the width of the filter vary with mass, becoming wider at higher masses at which peaks are wider. Typical values for this unit are 0.1–1.0. See “*The Mass Units page*” on page 70 for a description of how the expected peak width is calculated. This filter is most effective over the full mass range and is thus the default filter.
- **Savitsky-Golay:** a good choice for preserving peak shapes of narrow peaks. If the main peaks of interest in the spectra are below an M/Z of approximately 2500, this filter is appropriate. Only **Constant Daltons** or **Constant, Points** are allowed as width units for this filter.
- **Signal enhance:** accentuates the lower, flatter peaks common to the high mass range. The underlying data is unaffected, however signal noise statistics can change. Signal enhance can be switched on and off. It is only available with the Average Filter option.
- **Pattern Pre-filter:** if active, removes point-to-point “saw-like” noise that can be part of the spectrum data, especially if the data are collected at 500 MHz or above. By default, it is a 4-point pattern period filter.

The Area page

The **Area** page determines how the areas of peaks are calculated, giving you the choice of indirect, slope based, or direct calculation.

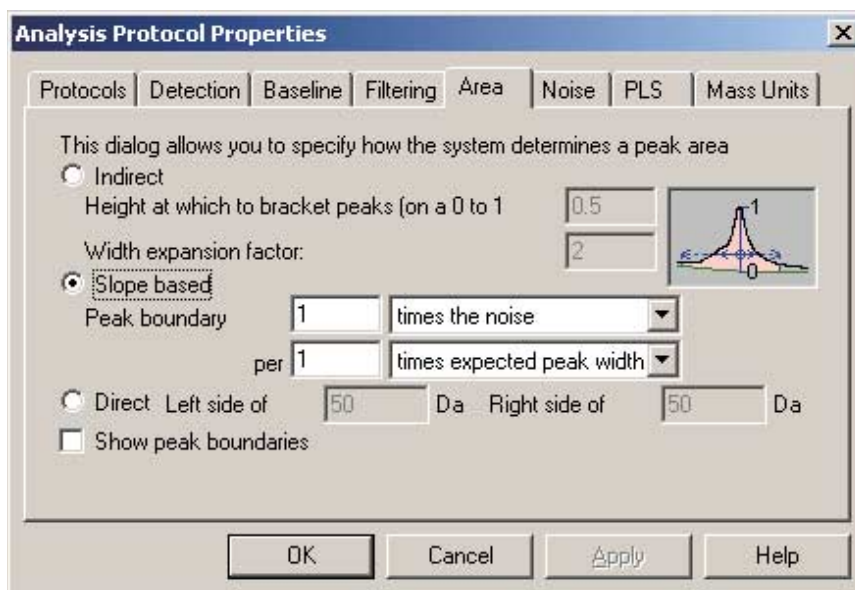


Figure 4-10: The Area page of the Analysis Protocol Properties dialog.

- **Indirect:** this option finds the area by measuring the peak width at the specified height from the baseline and multiplying it by the chosen **Width expansion factor** (slope-based or direct, see below).
- **Slope based:** this method finds the peak boundaries by measuring the slope of the spectrum data and determining when it is less than the specified value. This option works well with resolved peaks that have signal to noise ratios larger than 10.
- **Direct:** allows the peak width to be set to a specific width, in daltons. This option is useful if you have one peak that you wish to estimate the area of in a spectrum, and the other methods are unsuccessful, usually because the peak has a low signal-to-noise ratio or overlaps another peak.
- **Show Peak Boundaries:** fills the peak area with color. This option can slow display of the spectrum and is not recommended if the spectrum is being displayed in the **Data Average** window.

The Noise page

The **Noise** page (Figure 4-11) controls how the noise values are calculated throughout the spectrum.

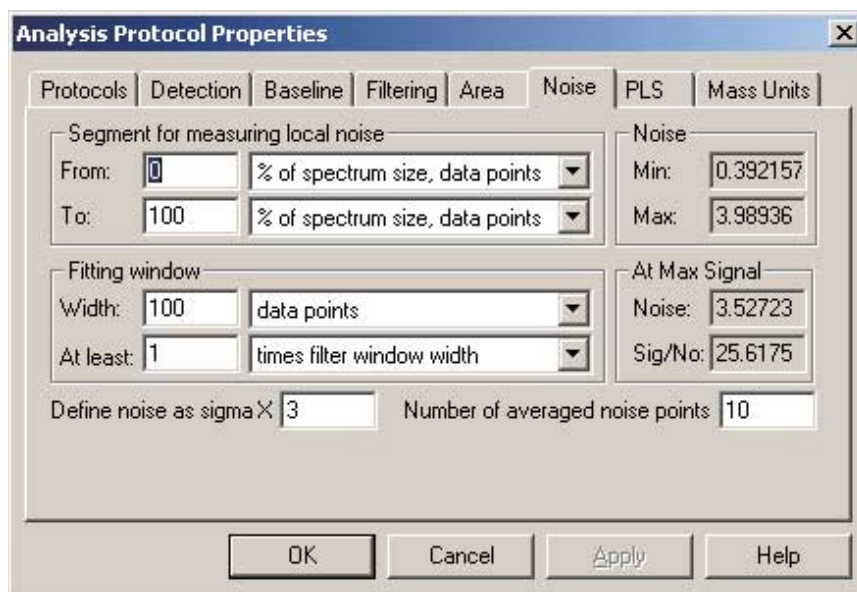


Figure 4-11: The **Noise** page of the *Analysis Protocol Properties* dialog.

- **Segment for measuring local noise:** defines the spectrum region for the noise calculation. Typically, the matrix (EAM) portion of the mass range is excluded from noise calculation. The units can be **% of spectrum size, data points**; **% of spectrum size, Daltons**; and **Daltons**.
- **Fitting window:** these parameters control the window width of each noise segment measured. The width can be specified in units of **times the expected peak width, Daltons** or **data points**. A typical range of values for this parameter is 50 to 100 data points.
- **Define noise as sigma X:** the standard deviation of the points in a segment are calculated and multiplied by this value to give an estimate of noise for this segment. Typically a value of 3 works well.
- **Number of averaged noise points:** since the estimate of the noise is highly variable for any one measurement, several measurements are averaged to improve the quality of the estimate.

The PLS page

The **PLS** page is used to specify rules for identifying amino acids from Protein Ladder Sequencing data.

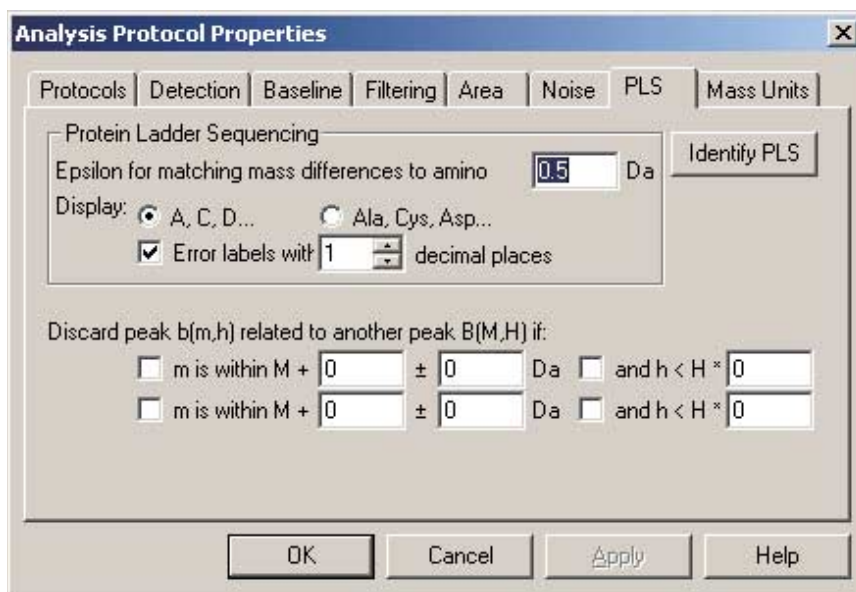


Figure 4-12: The **PLS** page of the *Analysis Protocol Properties* dialog.

- **Epsilon for matching mass differences to amino acids:** determines how large a window is used when finding candidate PLS amino acids.
- **Display:** has options for displaying the sequence as single letter amino acid abbreviations or 3 letter codes.
- **Discard peak:** contains settings that are rules for ignoring specific mass relationships so that adducts do not interfere with PLS identification.

The Mass Units page

The **Mass Units** page (Figure 4-13) defines the values used to estimate peak widths and mass errors. Since these values vary over the mass range, tables are used to define the values at specific masses, and the value at a given mass is calculated by linear interpolation.

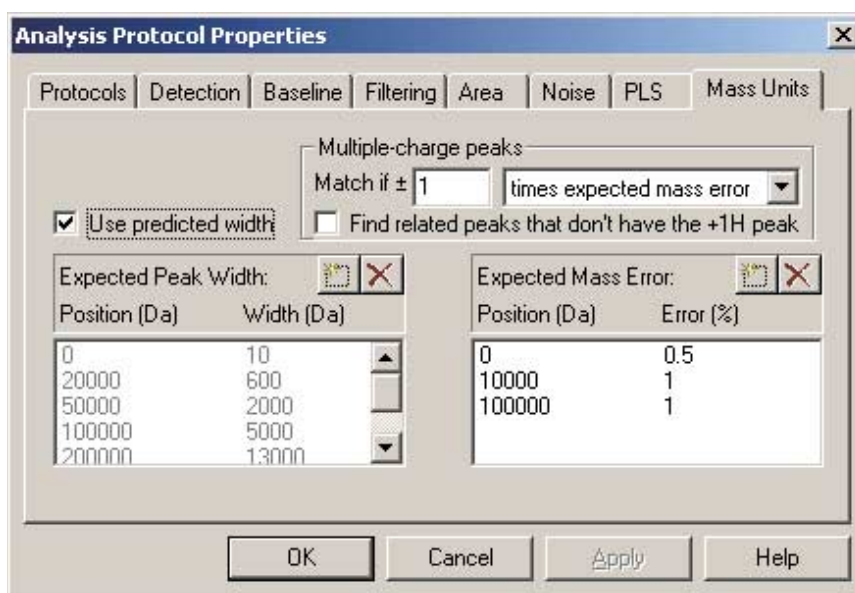


Figure 4-13: The **Mass Units** page of the **Analysis Protocol Properties** dialog.

- **Use predicted width:** if this option is checked, peak widths are predicted from the peak broadening factor, instead of using the **Expected Peak Width** table.
- **Multiple-charge peaks:** these options control the mass tolerance when finding multiply-charged peaks.
- **Expected Peak Width:** used when a width unit is required.
- **Expected Mass Error:** used if mass tolerance values are required, for example in the multiple-charge peak determination.

Chapter 5: Experiments

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Managing experiments

Experiments are a powerful way of displaying and analyzing groups of data files.

Opening experiments



1. To open an experiment, select **Open | Experiment** from the **File** menu or click the **Open Experiment** button on the **Standard** toolbar. This will bring up the **Open Experiment** dialog, allowing you to open experiments that have been saved in the current database.

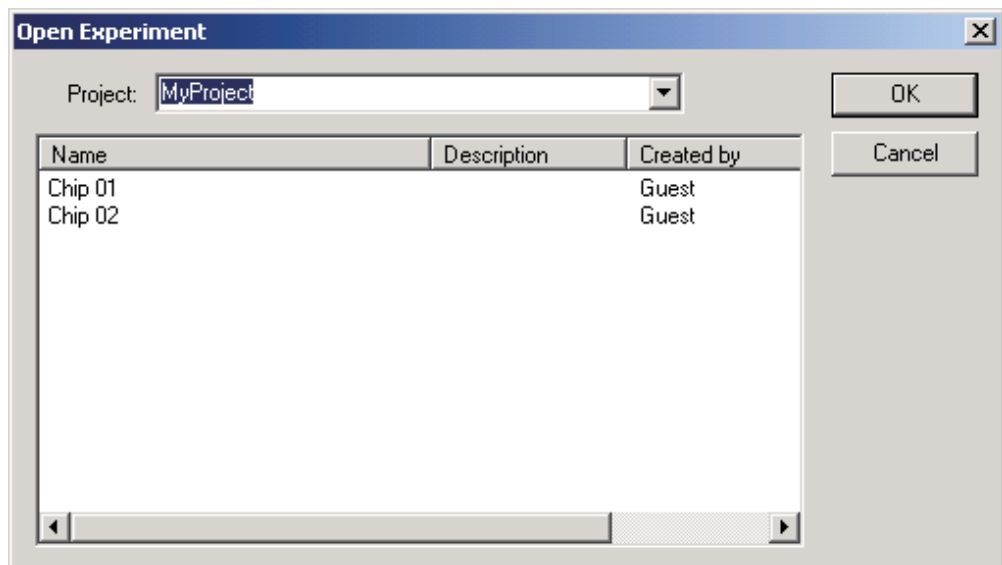


Figure 5-1: The *Open Experiment* dialog.

2. In the **Open Experiment** dialog, first select the project containing the experiment(s) you want to open, then select one or more experiments from the list. You can select multiple experiments by using the <Shift> (for contiguous selections) or <Ctrl> (for discontinuous selections) keys. You can sort the list of experiments by single-clicking the column headers.

Importing single experiments

The **Open Experiment** dialog only allows you to open experiment files saved in the current database. If you want to use files stored in another database, or perhaps a file sent by a colleague as an .xpt file, you will need to import the experiment files to the current database. Experiments can be imported singly, or in batches. The steps below outline how to import a single experiment.

1. Select **Import | Experiment (.xpt)...** from the **File** menu. The **Import file** dialog will open.

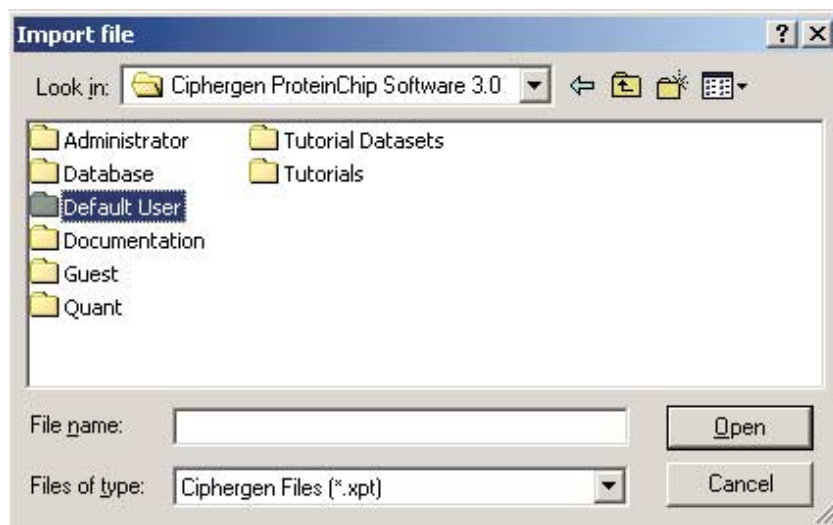


Figure 5-2: The Import file dialog.

2. In the **Import file** dialog, browse to the *.xpt file you wish to open.
3. Note that if you want to add the experiment to the current database, you must explicitly save it into the database (see "Saving experiments" on page 77).

Batch importing experiments

The ProteinChip Software allows batch-importing of files for convenience.

1. Select **Batch...** from the **File | Import** menu to open the **Batch Import and Save to Database** dialog (Figure 5-3).

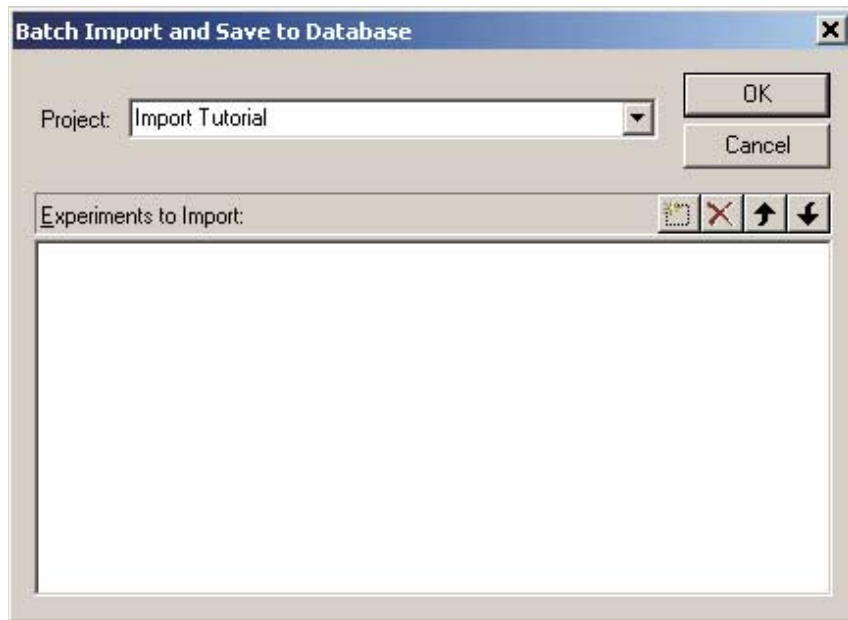


Figure 5-3: The *Batch Import and Save to Database* dialog.

2. From the **Project** drop-down menu, select the project into which you want to import the files.
3. Press the **New (Insert)** button to begin selecting files to import. The ... button will appear below the down arrow button.


New (Insert)
button

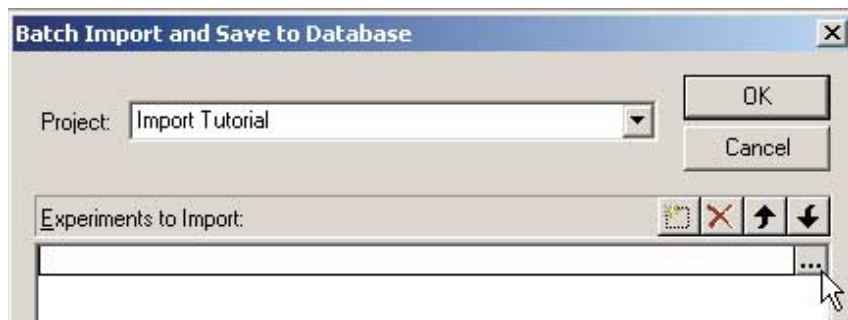


Figure 5-4: Clicking the **New** button in the *Batch Import and Save to Database* dialog causes the... button to appear.

4. Click the ... button to open the **Select Experiments to Import** dialog (Figure 5-5).



Figure 5-5: The *Select Experiments to Import* dialog.

5. Use the dialog to browse to the directory containing the experiment files you wish to import.
6. Select the spectra to be imported by highlighting them, holding down the <Ctrl> key to select multiple spectra as desired. Press **Open** to load the files into the **Batch Import and Save to Database** dialog.

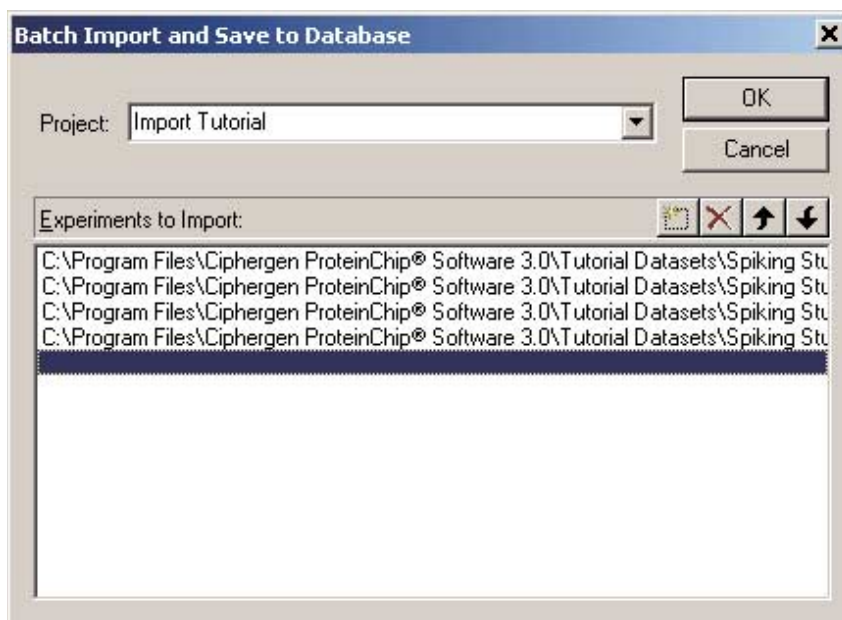


Figure 5-6: The *Batch Import and Save to Database* dialog, with files to be imported.

- Once the files are loaded, press **OK** to begin the batch import. Each file is loaded into ProteinChip Software 3.0 and then automatically saved into the selected project in the database.



NOTE: See the “Data management tutorial” on page 35 for a step-by-step tutorial on batch-importing experiments into databases.

Saving experiments

- To save an experiment, select **Save** from the **File** menu. If the experiment was opened from the current database, it will automatically save any changes into the database. If you want to save an experiment to a different project or with a different experiment name, select **Save As...** from the **File** menu to open the **Save Experiment** dialog (Figure 5-7).
- If the experiment was imported to the current database, or if you are using **Save As...** to save it with a different name or to a different project, choosing **Save** or **Save As...** from the **File** menu will open the **Save Experiment** dialog (Figure 5-7).

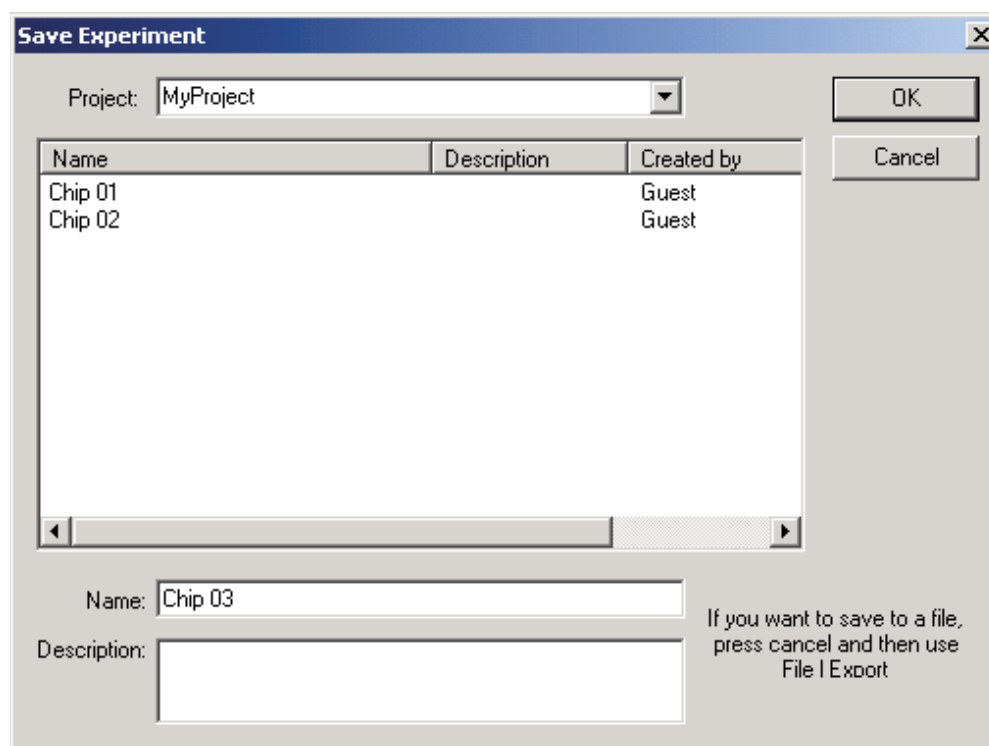


Figure 5-7: The **Save Experiment** dialog.

- In the **Save Experiment** dialog, select the project you wish to save the experiment into from the **Project** dropdown list.

4. Enter an experiment name in the **Name** field. The default experiment name will be based on the original.xpt filename.
5. You may optionally enter a description of the experiment.
6. When saving an experiment that contains copies of spectra that already exist in the project you are saving to, the **Overwrite sample props?** alert will appear (Figure 5-8). The alert allows you to choose which sample properties to keep. To keep the sample properties already in the database, select **No** or **No to All**. If you wish to overwrite the sample properties in the database with the sample properties in the spectra you are saving to the database, select **Yes** or **Yes to All**. Selecting **No to All** or **Yes to All** will answer **No** or **Yes** for all the spectra that have this conflict.

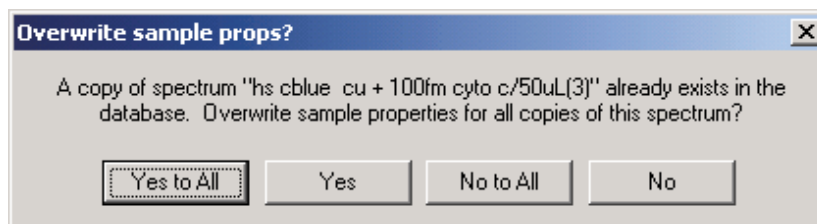


Figure 5-8: The *Overwrite sample props?* alert.

Changing experiment names

1. The name of the current experiment can be changed by selecting **File | Properties | Experiment....** The **Experiment** dialog will open.

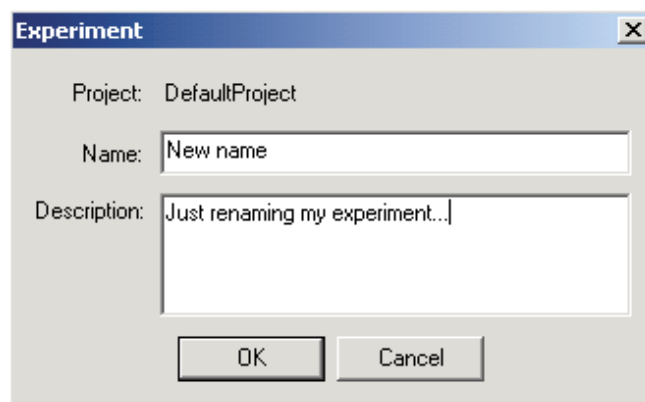


Figure 5-9: The *Experiment* dialog.

2. Enter a new name and change the experiment description as desired. Click the **OK** button to implement the changes. If you have entered the name of an existing experiment in the current project, an alert will appear, prompting you to enter a different name.

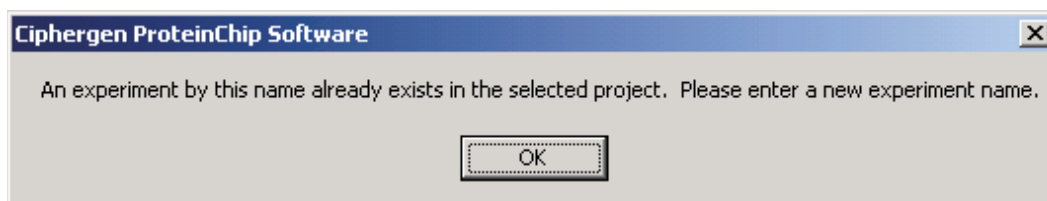


Figure 5-10: The alert that appears when experiment names conflict.

Deleting experiments

1. To delete an experiment, select **Delete | Experiments...** from the **File** menu. The **Delete Experiment** dialog will open.

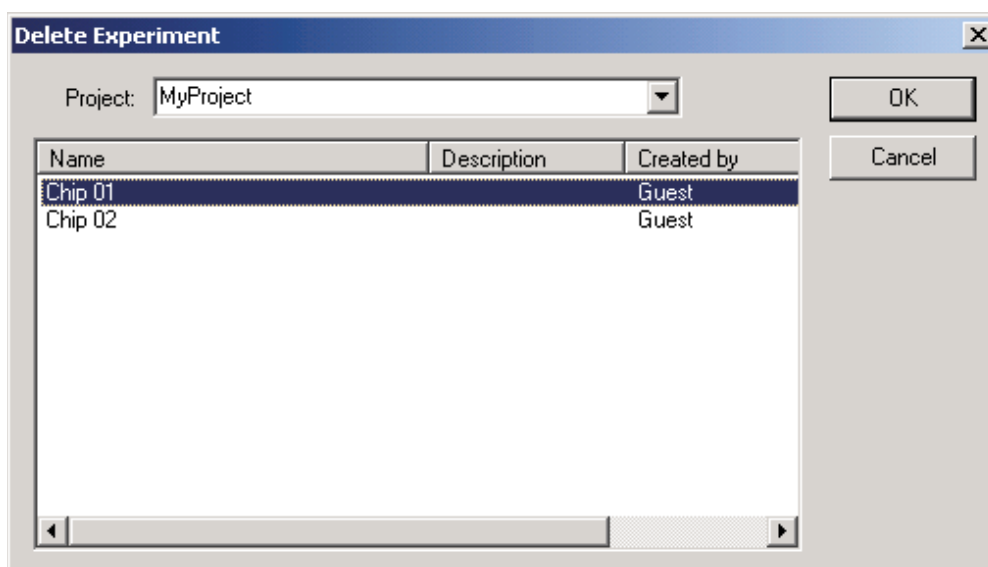


Figure 5-11: The Delete Experiment dialog.

2. Use the **Project** drop-down list to select the project from which you wish to delete an experiment, then select one or more experiments from the list. You can select multiple experiments by using the <Shift> key (for contiguous selections) or <Ctrl> key (for discontinuous selections). You may sort the list of experiments by single clicking the column headers. When you have finished selecting experiments, click the **OK** button.
3. An alert will appear, requesting you to confirm that you wish to delete the selected experiments (Figure 5-12). Click the **Cancel** button to be returned to the **Delete Experiment** dialog, or click **OK** to continue deleting the file(s).

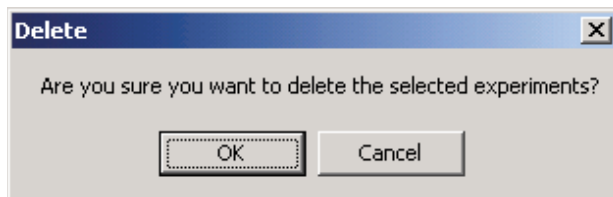


Figure 5-12: The Delete alert.

4. If an experiment you have selected to be deleted is currently open, you will not be able to delete it and an error message will appear (Figure 5-13).

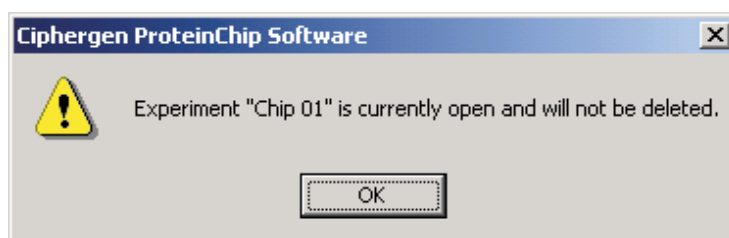


Figure 5-13: The error message that appears if an open experiment is selected for deletion.

Inserting spectra into experiments

1. Open an experiment, then select **Insert Item...** from the **Experiment** menu. The **Insert spectra** dialog will open. The dialog allows you to browse through the projects, experiments, and spectra associated with the current database. An experiment doesn't need to be open for you to insert its spectra into another experiment.

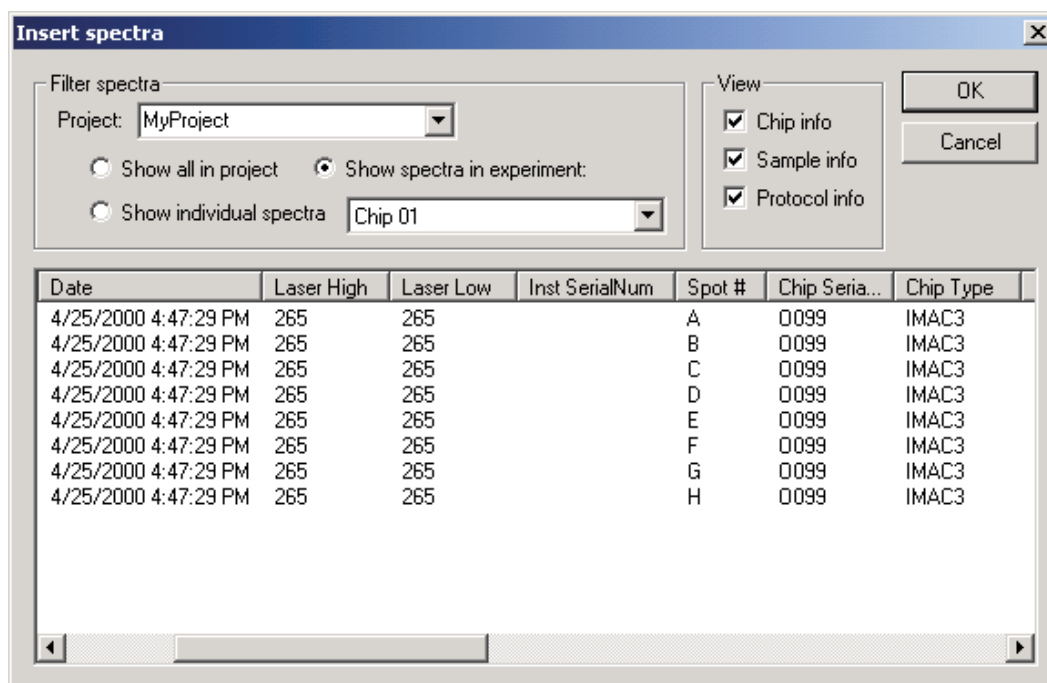


Figure 5-14: The *Insert spectra* dialog.

2. Use the **Filter Spectra** options to select the project, then select which spectra in the project you want to view. You may want to view all spectra contained in all experiments in the project, to view only the spectra contained in a particular experiment, or to view only the spectra saved as single spectra in the project.
3. Use the dialog's **View** options to select which spectra fields you want to view. You may want to view chip information including the chip serial number and type, the sample information including sample group (important when using the Biomarker Wizard), or the protocol information including fraction, wash, and EAM information.
4. Select spectra from the list. You can sort the list of spectra by single-clicking on the column header of the field you wish to sort by. Clicking twice on a column header will toggle from ascending to descending order. You can select multiple spectra by using the <Shift> (for contiguous selections) or <Ctrl> (for discontinuous selections) keys.
5. When you have finished selecting spectra, click the **OK** button to insert the spectra you have selected into the current experiment.

Editing sample properties

1. Select **Sample Properties...** from the **Experiment** menu. The **Sample Properties** dialog will open (Figure 5-15).

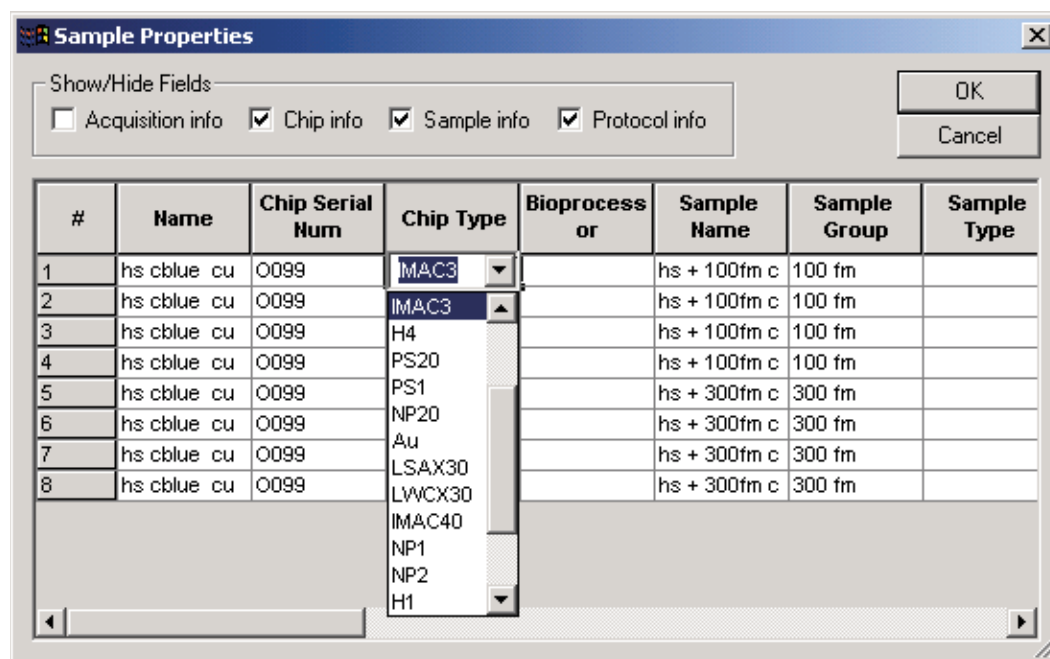


Figure 5-15: The *Sample Properties* dialog.

2. Use the **Show/Hide Fields** options to select which fields to show/hide. In the figure, the **Acquisition info** (laser intensity, acquisition date) is hidden.
3. You may edit any of the sample properties that are not read-only (the acquisition information is read-only). If the experiment you are editing is saved in the current database, you may select from a list of existing sample properties in the database (as shown for **Chip Type** in Figure 5-15).
4. After clicking the **OK** button, the sample properties will be cached with the experiment. They will not be saved unless you save the experiment.
5. For experiments that are stored in a database, the sample properties must remain consistent across all copies of a spectrum. While the analysis properties applied to multiple copies of a single spectrum may vary, the sample properties must remain consistent for all copies of the spectrum. See the previous section on saving experiments for more details.

Sorting the spectra in an experiment

1. Select **Sort Item...** from the **Experiment** menu. The **Sort** dialog will open.

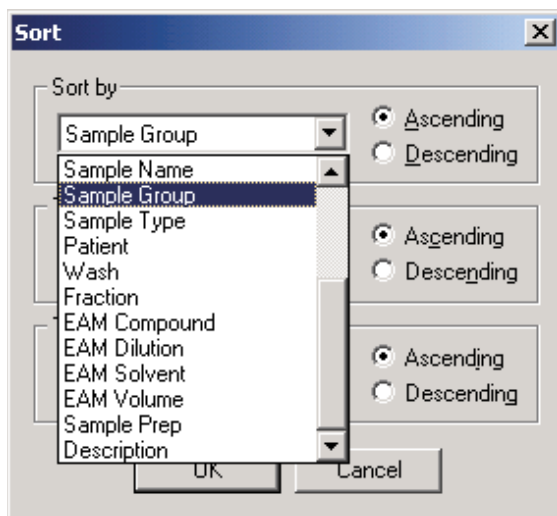


Figure 5-16: The Sort dialog.

2. Use the **Sort** dialog select the field(s) you wish to sort by. You may select up to a 3-level nested sort. The fields that you may sort by are the same fields as in the **Sample Properties** dialog.
3. Designate **Ascending** or **Descending** order for each level of sort.
4. When you have finished specifying the sort order, click **OK** to sort the spectra.

Working with experiments

Navigation tips for experiments

- To select a spectrum in an experiment, click on the spectrum tag to the right of the spectrum.
- To select all of the spectra in an experiment, press Ctrl-A.
- To de-select all of the spectra in an experiment, click outside the spectra and not on an item tag or an annotation.
- The analysis and presentation protocols work on groups of spectra in an experiment. Select the spectra to operate on before opening one of the protocol dialogs.
- Individual spectra can be inserted into an experiment using the clipboard. Select the spectrum window and press Ctrl-C to copy it. Select the experiment window and press Ctrl-V to paste a copy of the spectrum into the experiment. Spectra can also be copied and pasted between experiments.

- The **Mass Range** slider control appears at the top of an experiment file (Figure 5-17). This slider allows you to easily traverse the entire mass range. The width of the mass window is determined by the current zoom range — simply move the slider back and forth to explore the mass range.



Figure 5-17: The **Mass Range** slider control.

- Using the mouse, it is possible to change the intensity scale or traverse the mass axis using the current zoom settings. The mouse pointers change to arrows. To change the scale/traverse the axis, click and drag in the direction of the arrow.

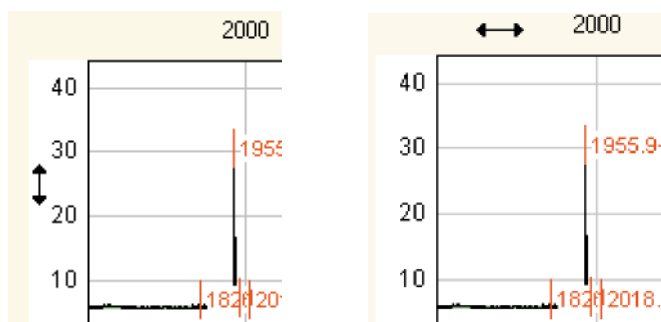


Figure 5-18: Changing the intensity scale (left) or traversing the mass scale (right) with the mouse.

- To set the scale for one or more spectra, select the spectra and right click on one of the spectrum tags. Select **Scales...** from the popup menu. You may also select **Scales...** from the **Spectrum** menu. Use this option if you wish to view a group of spectra using the same intensity scale. The **Scale Settings** dialog will open (Figure 5-19). In the dialog, un-check **Autoscale** to view a group of spectra on the same intensity scale. You can use the **Intensity** and **Mass Range** options to manually adjust the scale.

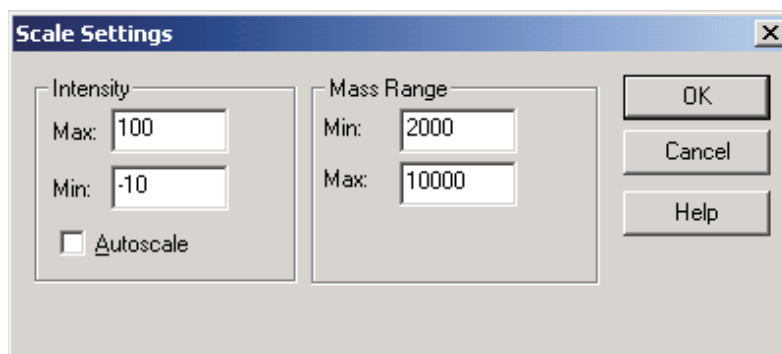


Figure 5-19: The **Scale Settings** dialog.

Spectrum views

Three spectrum view types are available — **Trace View**, **Gel View**, and **Map View**. The views buttons on the **Data Analysis** toolbar can be used to toggle between the view types.

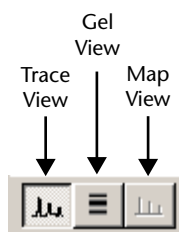


Figure 5-20: The Trace View, Gel View, and Map View buttons.

- **Trace View** is the traditional mass spectrometer display. It is useful for identifying and characterizing peaks in the spectrum.
- **Gel View** (the peaks are represented by dark bands, similar in appearance to a stained electrophoretic gel) is often used for comparing multiple spectra.
- **Map View** is used to reduce the clutter when working with larger numbers of peaks. Only peaks that have been labeled, or “centroided,” are displayed in **Map View**, represented as thin vertical lines.

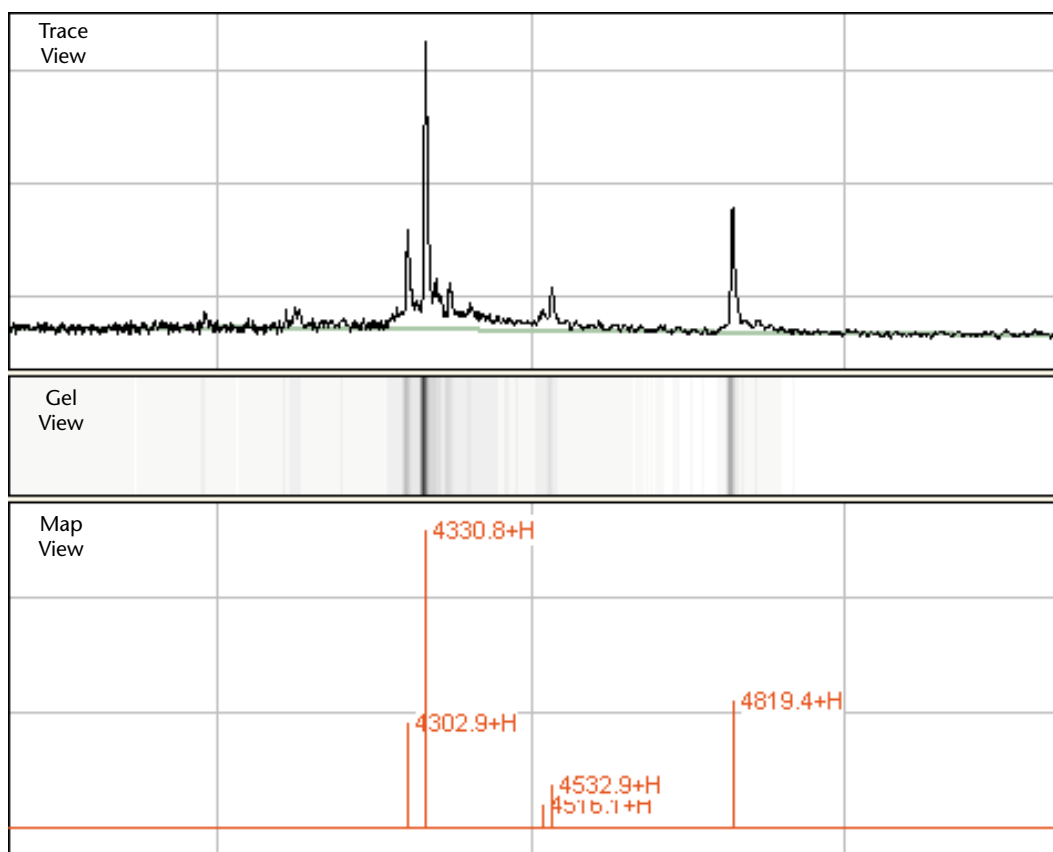


Figure 5-21: Data displayed in **Trace View** (top), **Gel View** (middle), and **Map View** (bottom).

Using the Experiment toolbar

The **Experiment** toolbar is anchored to the experiment window's frame. It contains controls for some of the most common experiment operations.

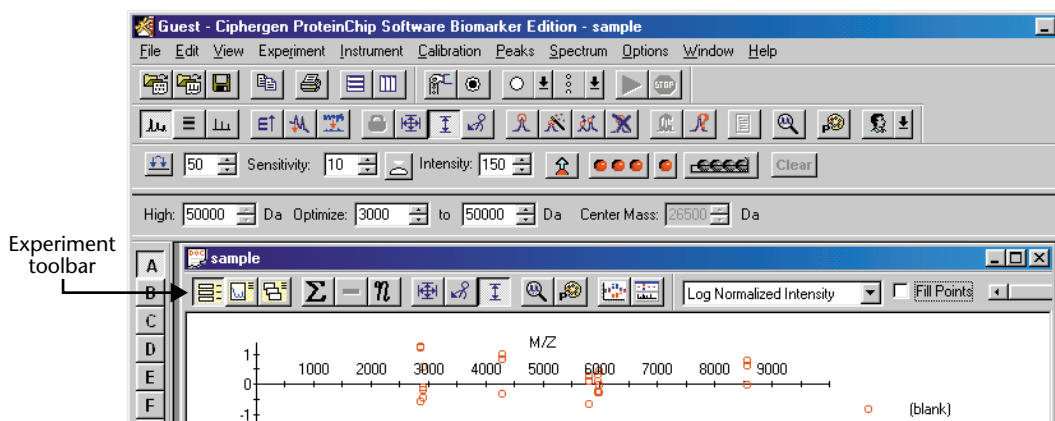
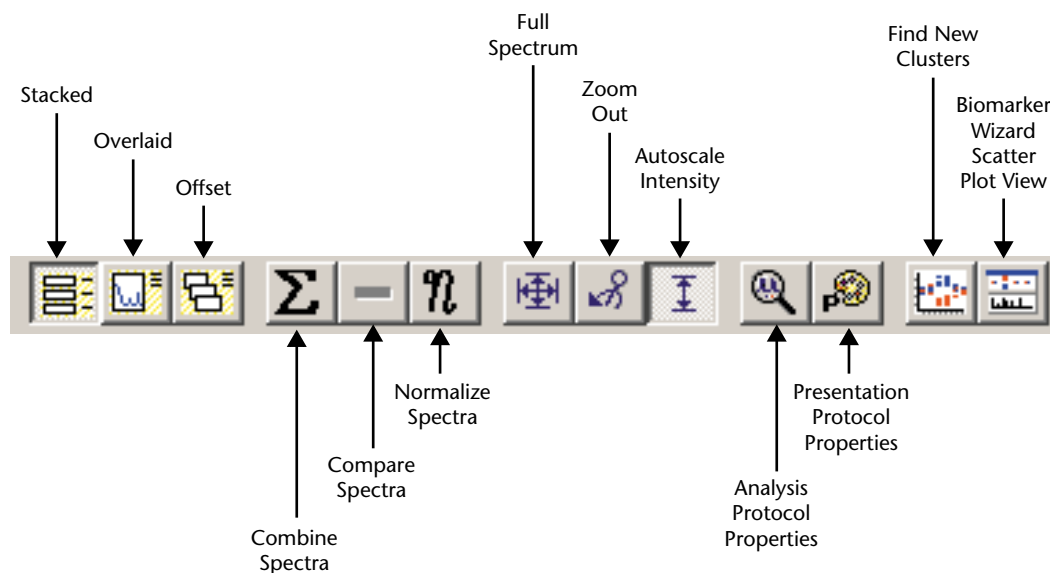


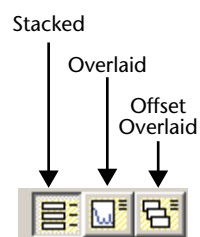
Figure 5-22: The **Experiment** toolbar is anchored to the top of the experiment window.



*Figure 5-23: The **Experiment** toolbar buttons.*

Spectra layouts

The experiment's spectrum layout is selected using the **Stacked**, **Overlaid**, and **Offset Overlaid** buttons in the **Experiment** toolbar.



*Figure 5-24: The **Stacked**, **Overlaid**, and **Offset Overlaid** buttons.*

- **Stacked:** is the default layout and displays the spectra aligned by molecular weight and stacked sequentially below each other as shown in Figure 5-25.

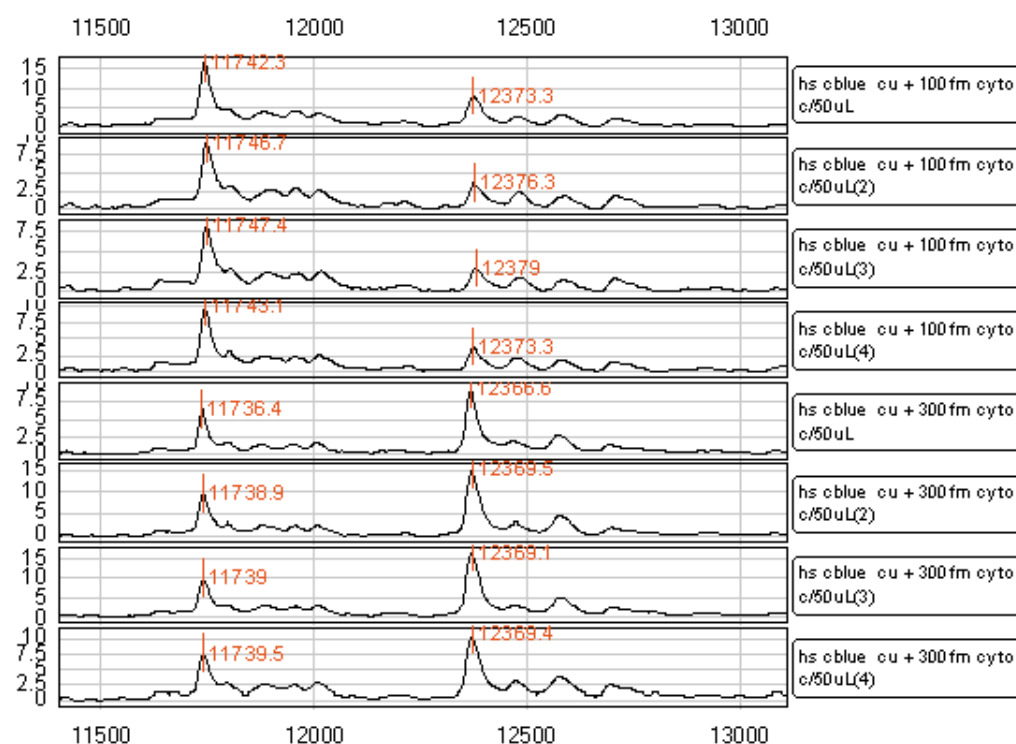


Figure 5-25: Data displayed in *Stacked* layout.

- **Overlaid:** spectra share a common X- and common Y-axes as shown in Figure 5-26. This layout is useful for showing differences between two or three spectra, but can become cluttered when comparing larger numbers of spectra.

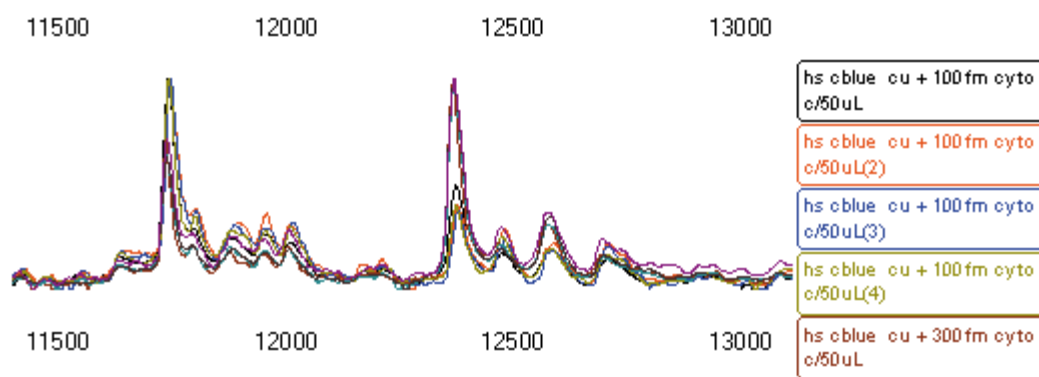


Figure 5-26: Data displayed in *Overlaid* layout.

- **Offset Overlaid:** is similar to **Overlaid**, except that the data are arranged in a three-dimensional manner, with the molecular weights offset by a few thousand daltons (Figure 5-27).

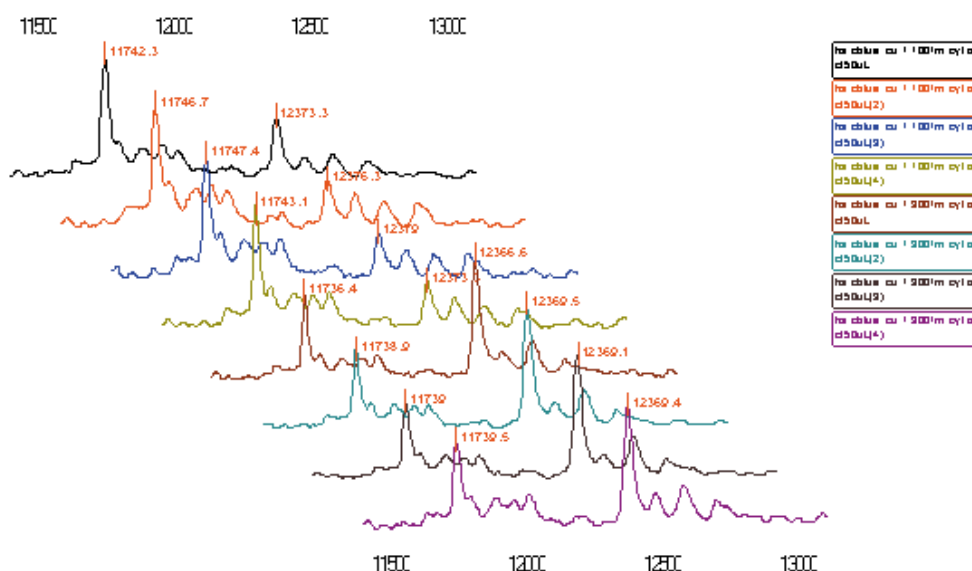


Figure 5-27: Data displayed in **Offset Overlaid** layout.



NOTE: Gel views are not compatible with the **Overlaid** or **Offset** layouts.

Creating peak maps

It is often desirable to compare data obtained from multiple spots; for example controls vs. experimental samples. Direct comparison of the data can quickly highlight differences in peak heights/areas, or underscore the presence or absence of a protein. The three tools primarily used for rapid data comparison are accessed via the **Combine Spectra**, **Compare Spectra**, and **Normalize Spectra** buttons.

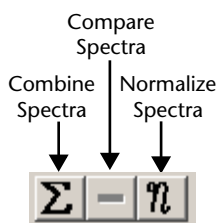


Figure 5-28: The **Combine Spectra**, **Compare Spectra**, and **Normalize Spectra** buttons.

- **Combine Spectra:** combines 2 or more spectra into a peak map.
- **Compare Spectra:** compares two spectra or peak maps.
- **Normalize Spectra:** normalizes the intensity or mass of two or more spectra.

The general procedure for using these analysis tools is:

1. Label the peaks of interest in the samples to be analyzed.
2. Select two or more spectra files by clicking on their sample tags (hold down the <Ctrl> key to select multiple spectra).
3. Click the appropriate button to compare the spectra.



NOTE: Always ensure that your data is properly normalized before combining data or comparing data. Incorrectly normalized data can present comparison artifacts (see below for details of normalizing data).

When comparing or combining spectra, a peak map of all the labeled peaks is created. This map contains the details about the peaks as they were at the time the map was created. Changes to the underlying peaks and spectra are not reflected in the peak map — it must be recreated to reflect the updated data.



TIP: When multiple peaks within the same spectrum are matched, a small diamond is shown at the base of the peak to indicate a problem. You can reduce the matching window or realign the peaks to avoid this problem.

Peak maps contain matching options to determine which peaks should be considered the same across different spectra. Flexible plotting and color options allow peak maps to be used in many ways.

Combining spectra

The **Combine Spectra** option creates a peak map by combining two or more spectra using matching options that you specify. To create a combination map:

1. Label all peaks from two or more spectra. Select the labeled spectra that you want to combine by clicking on the sample tag to the right of each spectrum (hold down the <Ctrl> key to select multiple spectra). At least two spectra must be selected to create a combination map.



NOTE: There is no limit to the number of spectra that can be combined into a single, representative protein profile.

2. Click the **Combine Spectra** button (refer to Figure 5-28), or select **Combine Spectra...** from the **Experiment** menu. The **Peak Map Options** dialog will open (Figure 5-29).

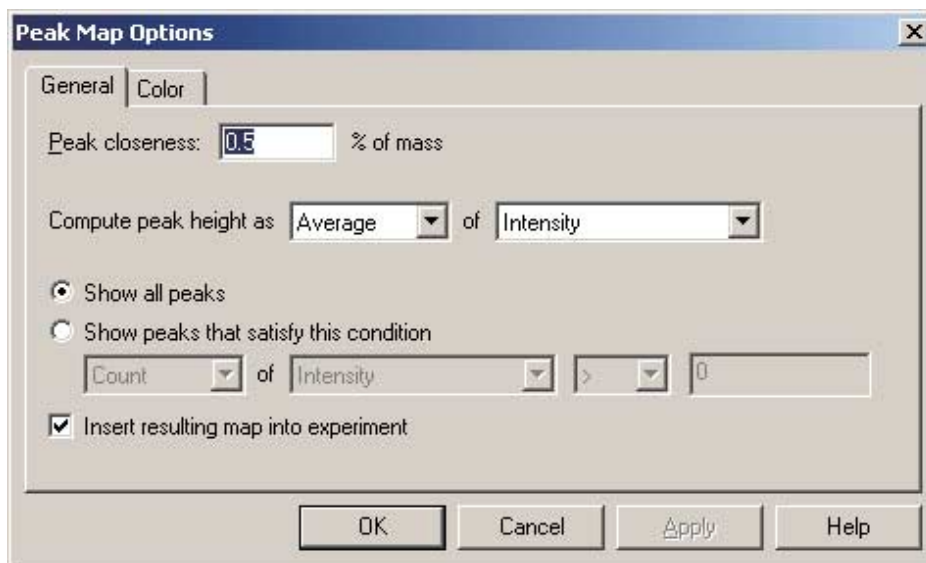


Figure 5-29: The *Peak Map Options* dialog.

3. Specify the **Peak closeness** (typically 0.5%) as well as how you want to compute the peak height (average vs. sum, intensity vs. area, etc.), and the criteria for displaying peaks (all peaks or only peaks that satisfy conditions that you define). Note that by default the peak map will be inserted into the experiment.

Comparing spectra

Comparison maps are a special case of combination maps that contain exactly two spectra to compare. To create a comparison map:

1. Label all peaks within two spectra. Select the labeled spectra that you want to compare by clicking on the sample tag to the right of the spectrum. Hold down the <Ctrl> key to select the second spectrum.
2. Click the **Compare Spectra** toolbar button (refer to Figure 5-28), or select **Compare Two Spectra...** from the **Context** menu. The **Peak Map Comparison Options** dialog will open (Figure 5-30).

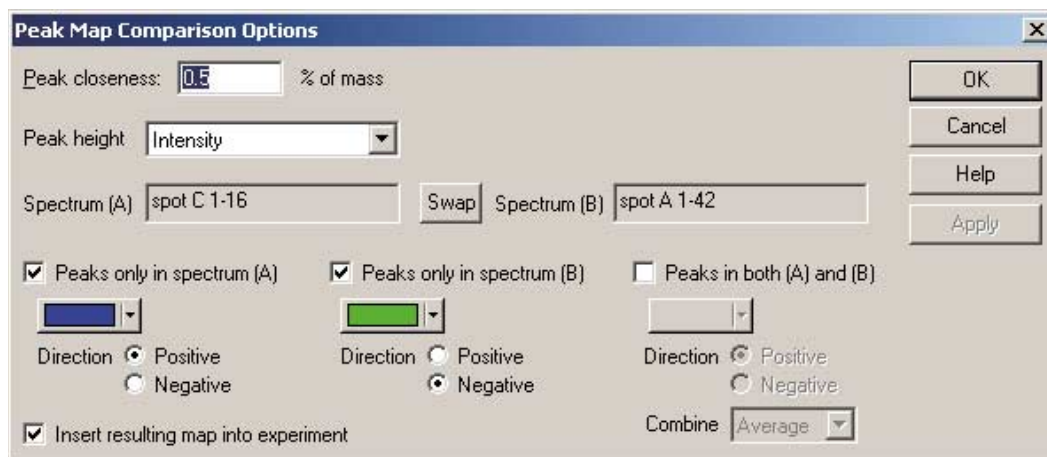


Figure 5-30: The *Peak Map Comparison Options* dialog.

3. Specify the **Peak closeness** (typically 0.5%) as well as how you want to compare the **Peak height** data (intensity vs. area, etc.)
4. Select which peaks to plot and how to display the data (color schemes, directions of differences, how to combine peaks if displaying peaks from two spectra).

Only two “maps” can be compared at one time. However, this can include two spectra files, two combination maps containing multiple spectra files, or any combination thereof.



NOTE: For easy analysis of two treatments containing multiple replicates, use the comparison wizard. Simply select all relevant spectra files (hold down the <Ctrl> key to select multiple spectra) and choose **Comparison Wizard** from the **Experiment** menu. Organize the spectra according to the treatment window and follow the prompts. The Comparison Wizard first generates two combination maps, then produces a comparison of the two combinations.

Normalizing spectra

Normalizing spectra helps compensate for variations in sample concentrations loaded onto a ProteinChip spot or slight shifts in mass due to imperfections on the ProteinChip Array surface. The default normalization dimension (i.e., normalization mode) is **Intensity, Total Ion Current**. Spectra can also be normalized according to intensity as measured by height, by intensity as measured by area or by mass.

The choice of which normalization method to choose is not always clear. Ciphergen recommends using the **Total Ion Current** method, as it does not contain the inherent variability of a single peak. The **Total Ion Current** normalization method makes certain assumptions about your samples. It assumes that on average, the total number of proteins being expressed is constant across the samples being normalized. In typical profiling experiments where the samples are complex and proteins are bountiful, this

assumption is generally valid. In other types of experiments such as purification optimization experiments or in profiling experiments in which a sample group is expected to express fewer proteins due to a drug treatment (for example), one cannot make such assumptions.



NOTE: Intensity normalization should always be run for groups of spectra that have been collected under similar conditions. For example, the array type, laser intensity, type of matrix, and sample fraction should all be the same for all the spectra being normalized together.

Normalizing using **Total Ion Current**

1. In the experiment, select the spectra to be normalized.
2. Click the **Normalize Spectra** button, or right-click near the peak to be normalized then select **Normalize Peaks...** from the pop-up menu. Alternatively, select **Normalize...** from the **Experiment** menu. The **Normalize Spectra** dialog will open. The dialog is modal, displaying different options in the lower half of the dialog depending on the chosen **Normalize Dimension**.

 **Normalize Spectra** button

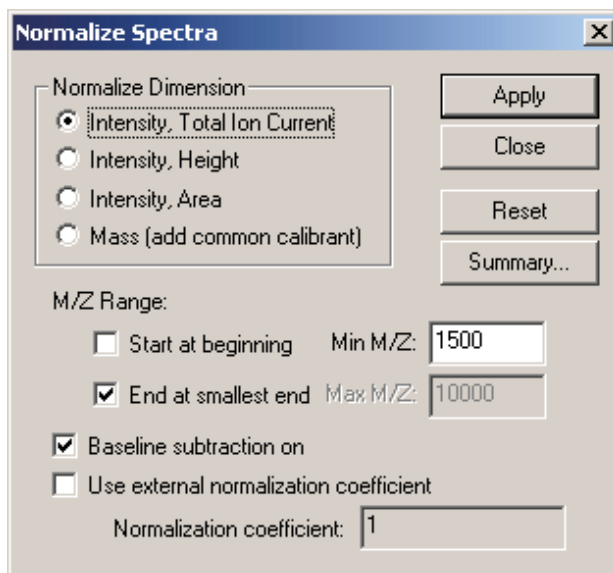


Figure 5-31: The **Normalize Spectra** dialog.

3. Select **Intensity, Total Ion Current** for the **Normalize Dimension**.
4. Select the **M/Z Range** over which to calculate the total ion current. The default is to start at an M/Z of 1500 but this value should be adjusted according to the type of matrix and laser intensity used to acquire the spectra. Make sure the M/Z range doesn't include any matrix signal that has been saturated. The default for the upper limit is to end at the smallest ending M/Z of all the spectra selected for normalization.

5. Select whether to turn on **Baseline subtraction**. The default is to turn it on.
6. Using an external **Normalization coefficient** normalizes spectra across experiments. If you have spectra you wish to normalize together in separate experiments, it is important to use the same the normalization coefficient. First, make sure the **M/Z Ranges** of the two spectra match. Normalize the first experiment. Copy the resulting **Normalization coefficient** to the clipboard. Open the second experiment. Turn on **Use external normalization coefficient** and paste in the **Normalization coefficient** from the first experiment.

Normalizing using a single peak height or area

In many cases it is difficult to know what mass to normalize against unless a specific internal standard is spiked into the sample. If an internal standard is not included, look for patterns of similarity between the protein profiles and normalize against a common, similar peak. If, after normalization, you find that all common peaks tend to be biased towards one sample, choose another peak to normalize against. Always ensure that your data is normalized properly before combining data or comparing data. Incorrectly normalized data can present comparison artifacts.

1. In the experiment, select the spectra to be normalized.
2. Make sure that the peaks that will be used have been labeled, either automatically or manually.
3. Click the **Normalize Spectra** button, or right-click near the peak to be normalized then select **Normalize Peaks...** from the pop-up menu, or select **Normalize...** from the **Experiment** menu. The **Normalize Spectra** dialog will open.
4. Select either **Intensity, Height** or **Intensity, Area** for the **Normalize Dimension**. The options in the lower half of the dialog will be as shown in Figure 5-32.



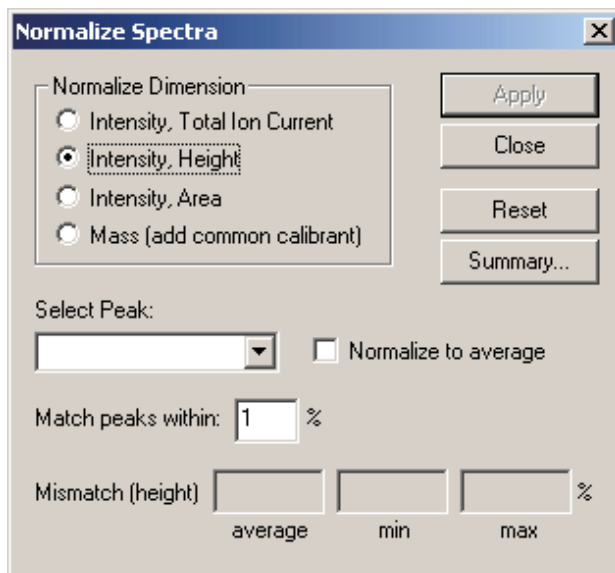


Figure 5-32: Using the **Normalize Spectra** dialog to normalize by peak intensity as indicated by height.

5. Use the **Select Peak** list to select the peak for normalization. This peak must be labeled in all of the selected spectra within the specified peak match window. Adjust the window to a larger mass range if needed.
6. Check the **Normalize to average** checkbox if you wish to normalize the intensities to the average of the peak intensities for the peak selected. If the checkbox is not marked, all spectra will be normalized to the first spectrum selected in the experiment.
7. When you have finished setting the dialog box options, click **Apply** to apply the specified normalization settings and return to the data plot.

Normalizing by mass

1. In the experiment, select the spectra to be normalized.
2. Make sure that the peaks to be normalized have been labeled, either automatically or manually. Mass normalization can only be used on labeled peaks.
3. Click the **Normalize Spectra** button, or right-click near the peak to be normalized then select **Normalize Peaks...** from the pop-up menu, or select **Normalize...** from the **Experiment** menu. The **Normalize Spectra** dialog will open.
4. Select **Mass (add common calibrant)** for the **Normalize Dimension**. The options in the lower half of the dialog will be as shown in Figure 5-33.



Normalize Spectra button

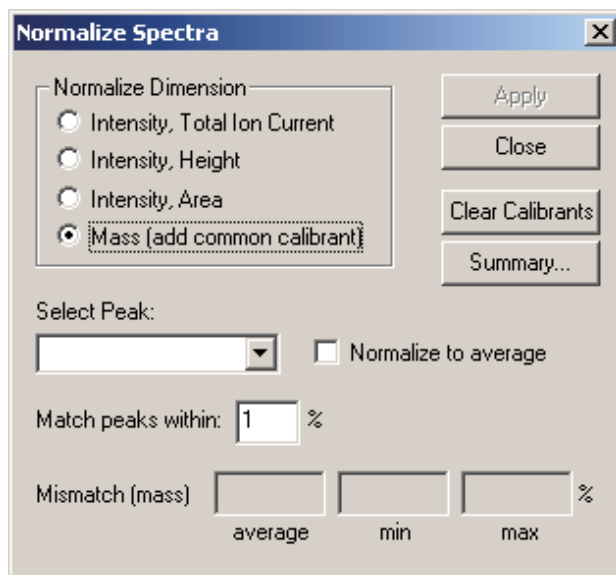


Figure 5-33: Using the *Normalize Spectra* dialog to normalize by mass.

5. Click the **Clear Calibrants** button to remove any previous internal calibration. Check the **Normalize to average** checkbox to use average masses for the masses of the internal calibrants.
6. In the **Select Peak** list, select the first peak to be used for normalization.
7. Click **Apply**.
8. Repeat steps 6 and 7 for any additional peaks. Using at least two peaks is recommended for accurate mass assignment.

Normalization summary

The last run intensity and mass normalization parameters and results can be viewed by either clicking the **Summary...** button in the **Normalize Spectra** dialog or by selecting **Normalization Summary...** from the **Experiment** menu.

Biomarker Wizard

Before starting Biomarker Wizard

- The first step in comparing data sets using Biomarker Wizard is to make sure that all spectra to be compared are in the same experiment. If the spectra are from different experiments, create a new experiment and populate it with the desired spectra using the **Experiment | Insert Item...** function.
- Once all the spectra have been put into a single experiment, spectra with similar conditions can be divided into sample groups (e.g., control or treated). Spectra are assigned to sample groups in the **Sample Properties** dialog, accessed via the **Experiment** menu, by simply typing in a sample group name

or choosing an existing sample group name from the list. Biomarker Wizard will use the sample group labels to generate statistical data from properly labelled sets of spectra.

- If you plan to normalize the spectra, do so before running Biomarker Wizard. Normalizing after running Biomarker Wizard will not properly update the peak intensities used when calculating statistics or generating Biomarker Patterns™ Software files. (Biomarker Patterns Software is an optional Ciphergen software product with a separate operation manual.)
- Biomarker Wizard requires at least two spectra to be selected before it can be used. If no spectra are initially selected, the software will ask if all spectra are to be selected.

Starting Biomarker Wizard



Find New
Clusters
button

Biomarker Wizard can be started by selecting the **Generate New Clusters...** menu item found under the **Experiment | Biomarker Wizard** menu, or by clicking the **Find New Clusters** button in the **Experiment** toolbar. Either action will open the **Biomarker Wizard — Generate New Clusters** dialog (Figure 5-34).

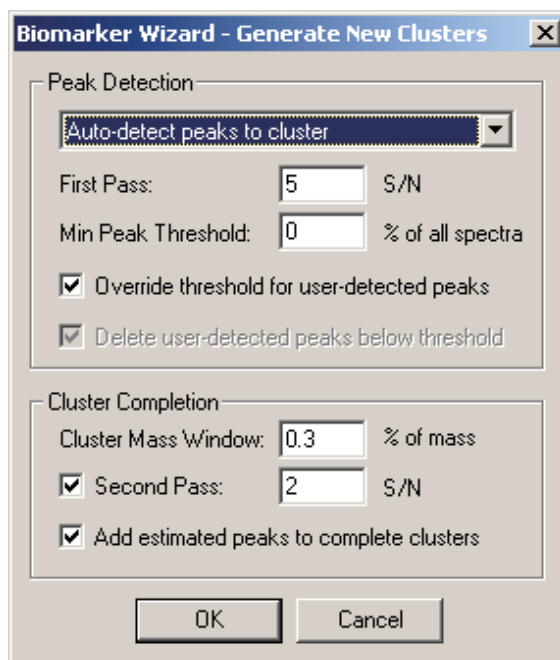


Figure 5-34: The **Biomarker Wizard — Find New Clusters** dialog.

Detecting peaks with Biomarker Wizard

Biomarker Wizard groups peaks of similar molecular weight from across sample groups of spectra, then statistically and visually displays differences in expression levels between sample groups. Like auto peak detection, Biomarker Wizard runs only on the currently displayed mass range.

Biomarker Wizard uses two separate peak detection passes. Pass 1 detects peaks that may be potential biomarkers, and forms clusters around them. Pass 2 attempts to populate the clusters from Pass 1 with peaks that were too small to be found in the first pass.

Peak detection options

1. Select the type of Biomarker Wizard run to perform from the **Peak Detection** drop-down menu: **Auto-detect peaks to cluster** or **Cluster only user-detected peaks**. The **Auto-detect peaks to cluster** setting will prompt the software to perform peak detection passes and automatically generate clusters. The **Cluster only user-detected peaks** setting causes the software to generate clusters around peaks that were detected before starting Biomarker Wizard. If the peaks of interest have been marked and **Cluster only user-detected peaks** is selected, proceed directly to the **Cluster Completion** section below.
2. Set the **First Pass** threshold. This determines the sensitivity of the first pass of detection. A lower value leads to a greater number of detected peaks and a greater number of clusters formed.
3. Set the **Min Peak Threshold**. This threshold is the minimum number of spectra, as a percentage of the total, in which a peak must be present in order to form a cluster. A cluster will not be formed around a peak if it is not present in the requisite number of spectra, and the label will be deleted. The **Min Peak Threshold** also affects the number of clusters formed.
4. Select **Override threshold for user-detected peaks** to cluster user-detected peaks during the automatic detection. If deselected, user-detected peaks must reach the minimum intensity value specified in the **First Pass** field before they will be clustered. In addition, deselecting this option enables the choice of keeping or deleting user-detected peaks that are beneath the **First Pass** threshold.

Cluster completion options

5. **Cluster Mass Window** specifies the width of the mass window as a percentage of a peak's mass. This will determine the width of the cluster as a function of molecular weight.
6. Define the sensitivity of the **Second Pass** of automatic peak detection to adjust the height of peaks used to complete a cluster. Peaks from the second pass are added to spectra lacking peaks after the first pass.
7. If selected, **Add estimated peaks to complete clusters** will arbitrarily assign a label to a small peak in each spectrum that still lacks peaks after first- and second-pass peak detection have been attempted.
8. Click **OK**. Biomarker Wizard will use the settings in the dialog to generate clusters.

Viewing Biomarker Wizard plots

The Biomarker Wizard data plot is a splitter-window appended to the top half of the experiment and is saved along with the experiment. Each color used in the data plot corresponds to a different sample group. There are four views defined for Biomarker Wizard plots, accessed through the drop-down menu on the **Experiment** toolbar.

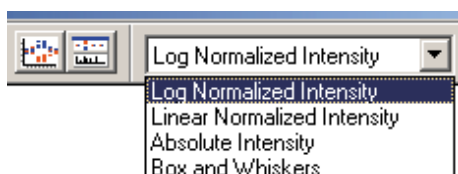


Figure 5-35: The drop-down menu of Biomarker Wizard plot views.

- **Log Normalized Intensity:** plots the log of peak intensity, normalizing the average intensity to 0. This view is useful because it expresses the differences in sample groups quite clearly, regardless of absolute intensity.

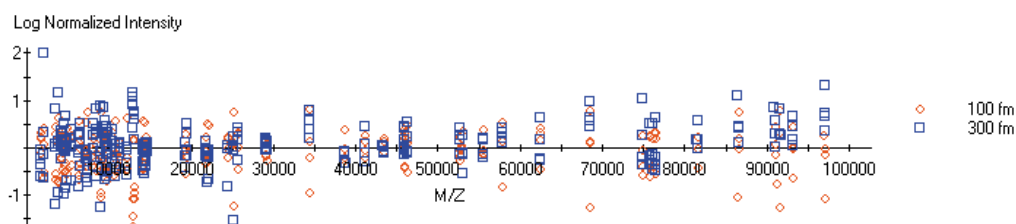


Figure 5-36: A Biomarker Wizard plot using log normalized intensity.

- **Linear Normalized Intensity:** normalizes the peaks in each cluster from 0 to 100 along the vertical axis.

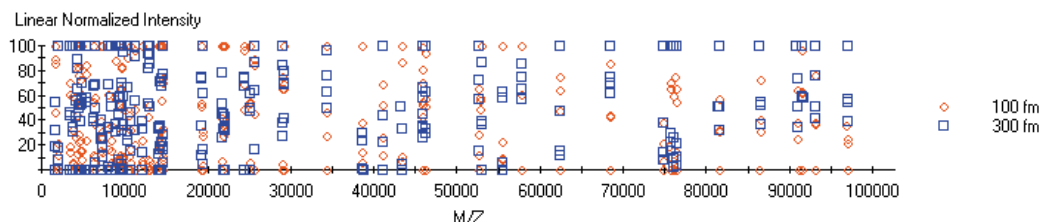


Figure 5-37: A Biomarker Wizard plot using linear normalized intensity.

- **Absolute Intensity:** displays all detected peaks by their absolute intensity and molecular weight

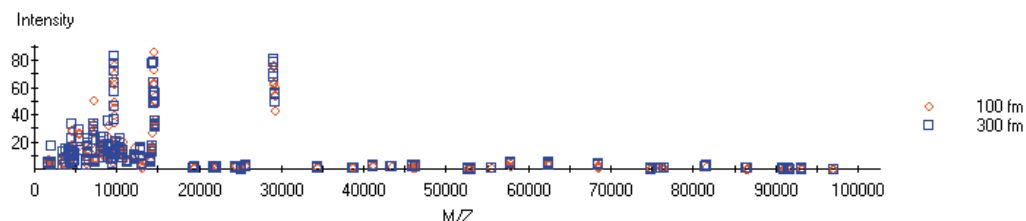


Figure 5-38: A Biomarker Wizard plot using absolute intensity.

- **Box and Whiskers:** this view transforms the contents of each sample group within a cluster into a box and whisker plot.

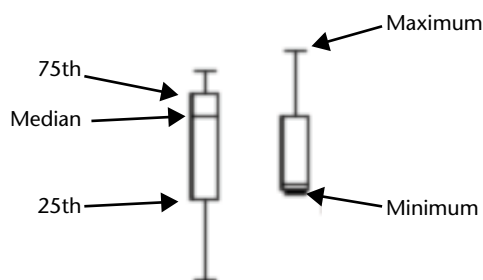


Figure 5-39: A Biomarker Wizard box and whiskers plot. The horizontal lines forming the boxes and whiskers indicate statistical averages for the sample groups.

Each box and whisker plot in the cluster represents data from one sample group. The boxes and whiskers indicate the maximum, the 75th percentile, the median, the 25th percentile, and the minimum peak intensity.

The view can be changed by selecting a view from the drop-down menu of views. The view can also be zoomed in and scrolled. Views will not dynamically update if peak data is changed after running Biomarker Wizard.

Hiding Biomarker Wizard plots

The **Biomarker Wizard Scatter Plot View** button toggles the view of the Biomarker Wizard plot on and off.

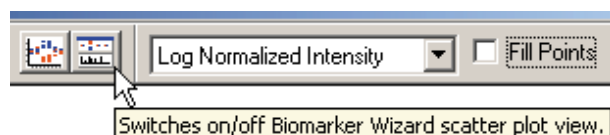


Figure 5-40: The Biomarker Wizard Scatter Plot View button.

Saving and loading clusters

Once a cluster list is generated, it can be saved and applied to other experiments.

To save a cluster list

1. Open the experiment containing the Biomarker Wizard-generated cluster list.
2. Select **Save clusters...** from the **Experiment | Biomarker Wizard** menu.
3. Select a destination for the file and specify a file name.
4. Click **OK**.

To load a cluster list

1. Open the experiment to be clustered.
2. Select **Load clusters...** from the **Experiment | Biomarker Wizard** menu.
3. Browse to the cluster list file (cluster lists end with ".ccl").
4. Click **OK**. The cluster list will be applied to the new experiment, and new peaks with a corresponding plot will be generated.

Viewing and exporting sample group statistics

To view p-values for each cluster

1. Select **Sample Group Selection** from the **Experiment | Biomarker Wizard** menu to open the **Sample Group Selection** dialog.

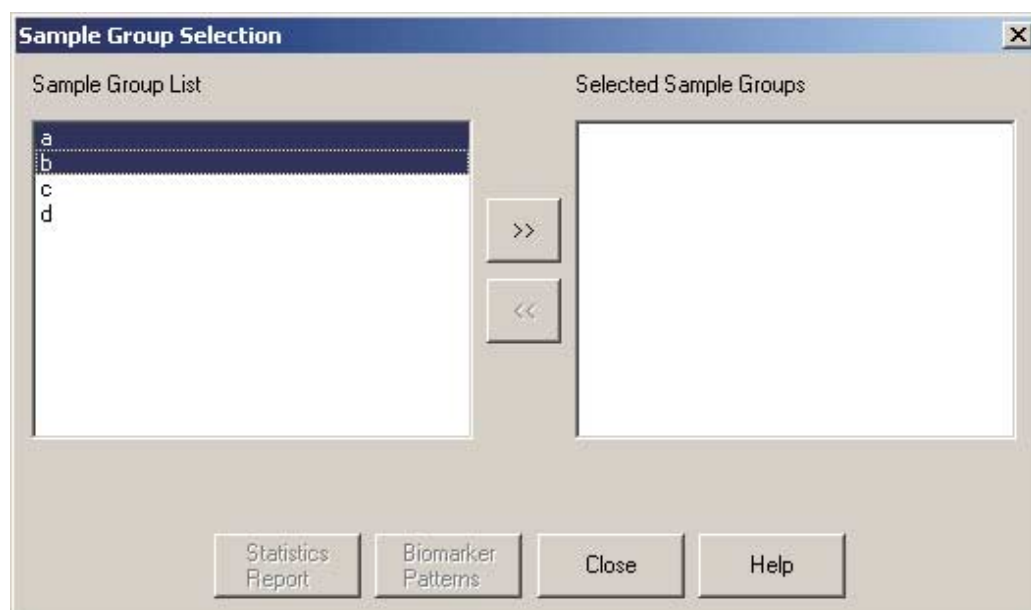


Figure 5-41: The *Sample Group Selection* dialog.

2. Select sample groups for comparison by highlighting names in the **Sample Group List** and clicking the >> button to transfer them into the **Selected Sample Groups** list. At least two sample groups must be selected.
3. Click the **Statistics Report** button. A chart showing the cluster average mass and p-values for each cluster will be displayed. These values can be saved to a file by clicking the **Export** button on the chart and specifying a file name for the exported data.

Exporting cluster information to Biomarker Patterns™ Software

1. Select **Sample Group Selection** from the **Experiment | Biomarker Wizard** menu to open the **Sample Group Selection** dialog (refer to Figure 5-41).
2. In the **Sample Group Selection** dialog, choose the sample groups to compare.
3. Click the **Biomarker Patterns** button.
4. Enter a file name for the data. The files are stored as *.csv files, which can be opened by Biomarker Patterns Software.

Chapter 6: Data Presentation

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Presentation protocols

Presentation protocols contain settings that affect the display of the data, such as the plotting style, the trace color and which items are included on the plot. The presentation protocol also controls experiment display and printing options.



NOTE: Additional display options are available for experiment views. See “Using the Experiment toolbar” on pages 86–87 for detailed information.



Presentation protocols are defined and saved using the **Presentation Protocol Properties** dialog. The dialog can be accessed by selecting **Presentation Protocol Properties** from the **Options** menu, or by clicking the **Presentation Protocol** button in the **Data Analysis** toolbar.

The Presentation Protocol Properties dialog

The Protocols page

The **Protocols** page allows you to create, save, and open presentation protocols, as well as setting protocols to the factory default values (Figure 6-1).

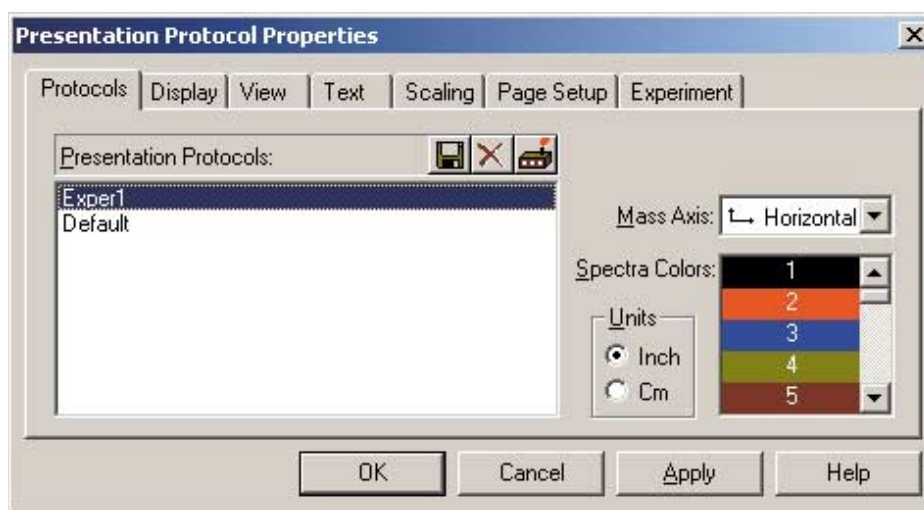


Figure 6-1: The **Protocols** page of the **Presentation Protocol Properties** dialog.

- **Presentation Protocols** list: click the name of a protocol to select it.
- **Save As** (small disk picture): opens a dialog that allows you to name and save the protocol.
- **Delete** (X): deletes the protocol selected in the **Presentation Protocols** list.
- **Factory Defaults** (small picture of a factory): resets the protocol to the factory default settings.
- **Mass Axis**: sets the orientation of the mass axis.

- **Spectra Colors:** change the default colors used when displaying multiple spectra. Double-click the number of the color you wish to change, then select a new color from the drop-down list of colors that will.
- **Units:** select the display units as inches or centimeters.

The Display page

The **Display** page controls the items displayed on the spectrum. When working with experiments, the **Show** section of the dialog is modal, displaying different options depending on the chosen spectra layout (stacked, overlaid, or offset overlay; see “Spectra layouts” on pages 87–89).

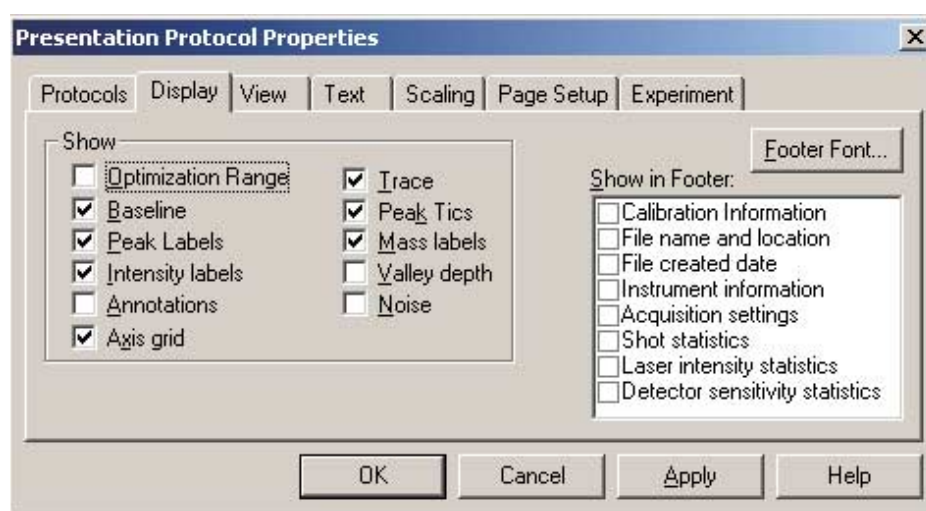


Figure 6-2: The **Display** page of the **Presentation Protocol Properties** dialog.

The View page

This page controls the view type, gel and trace color options, plot area margins and several drawing options described below.

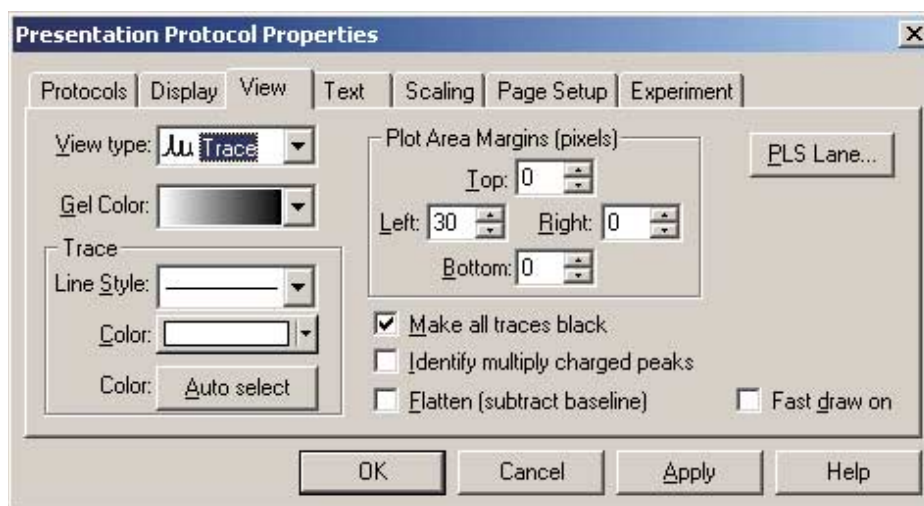


Figure 6-3: The **View** page of the **Presentation Protocol Properties** dialog.

- **View type:** allows selection of one of the three spectrum view types — **Trace**, **Gel**, and **Map**. **Trace** view is used when identifying peaks or looking for small features. **Gel** view is often used for comparing multiple spectra. **Map** view is used to reduce visual clutter when working with large numbers of peaks.
- **Gel Color:** gel views can be colored using several different color schemes.
- **Line Style:** several different line styles are available so that overlaid spectra can be differentiated when printed in black and white or copied to the clipboard.
- **Color:** this combo box selects the trace color for the selected spectra.
- **Color: Auto select:** makes automatic color choices for the selected spectra.
- **Plot Area Margins:** these values control the margin around the plot. When working with experiments, the top and bottom margins are normally set to 0 to avoid multiple sets of mass axis labels on the plot.
- **Make all traces black:** this option forces all of the traces to display as black.
- **Identify multiply charged peaks:** identifies multiply-charged peaks and displays them with the label style specified in the **Text** page (see below).
- **Flatten (subtract baseline):** subtracts the baseline for the display. This changes the plot only; it has no effect on the reported values or the raw data.
- **Fast draw on:** fast draw is convenient for quickly scanning a spectrum for peaks during data collection, and for increasing the data acquisition rate. It reduces the number of data points drawn to the maximum value at each horizontal pixel location.

The Text page

The **Text** page (Figure 6-4) controls the text properties such as color and orientation of the spectrum text items. The most commonly-used options are rotating the peak labels by 90 degrees by changing the orientation value to 90 and changing the vertical offset of the peak labels.

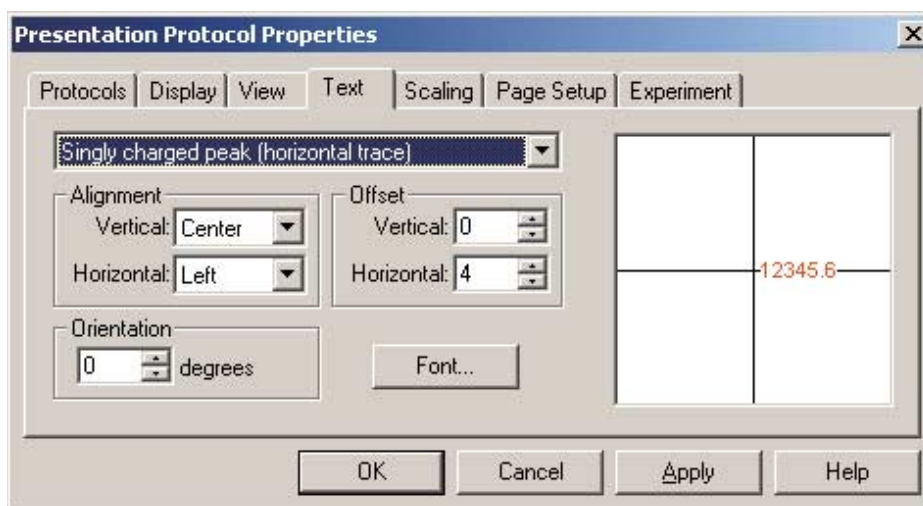


Figure 6-4: The **Text** page of the **Presentation Protocol Properties** dialog.

The Scaling page

The options on this page control the plot size and offsets used to display and print experiments and spectra.

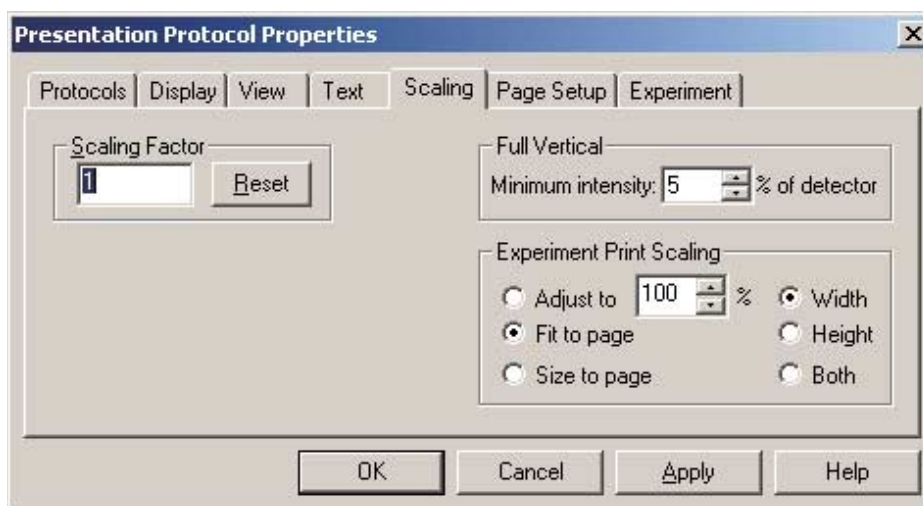


Figure 6-5: The **Scaling** page of the **Presentation Protocol Properties** dialog.

- **Scaling Factor:** a normalization factor used when intensities are normalized in experiment mode. Resetting this value to 1.0 returns the intensity scaling to its original value.
- **Full Vertical:** limits the smallest vertical scale that can be displayed.

The options in the **Experiment Print Scaling** section determine how the experiment is scaled to a printed page:

- **Adjust to:** allows you to manually set the percentage of the page size the spectrum will be printed at.
- **Fit to page:** will reduce the size of the spectra if required to fit them to a page.
- **Size to page:** will expand or reduce the size of the spectra to the size of the current page.
- **Width, Height or Both:** determine what portions of the experiment will be scaled to the printout size. Selecting **Both** prints all of the spectra on one page.

The Page Setup page



***NOTE:** The **Page Setup** page is modal, offering slightly different options for spectrum and experiment views. The options shown here are for experiment views.*

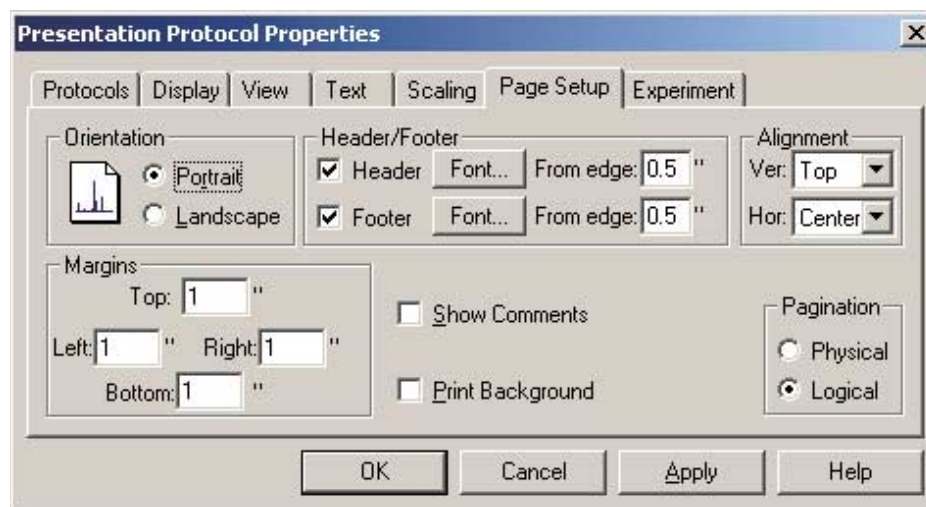


Figure 6-6: The **Page Setup** page of the **Presentation Protocol Properties** dialog.

- **Orientation:** toggles between **Portrait** and **Landscape** orientation for printing.

The **Header/Footer** options control the font and location of the header and footer text, as well as toggling display of the header and footer on and off.

- **Margins:** specifies the amount of margin space between the plot and the edges of the page.
- **Show Comments:** if selected, prints the spectra comments on a separate page.

- **Print Background:** determines if the screen background color is printed around the spectra. When using a color printer, turning this option off saves ink.
- **Pagination:** **Physical** pagination allows spectra to span page breaks. This can be useful when you want a spectra printout larger than a single sheet of paper. **Logical** pagination places page breaks between spectra.

The Experiment page

The **Experiment** page contains experiment-specific options for the presentation protocol. This page of the dialog is modal, displaying different options depending on the chosen spectra layout mode (stacked, overlaid, or offset overlay; see “Spectra layouts” on pages 87–89).

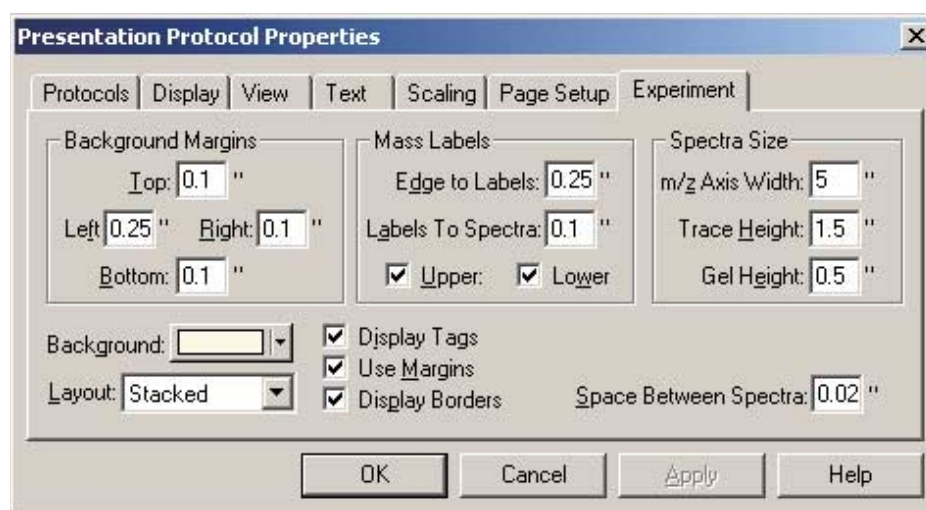


Figure 6-7: The **Experiment** page of the **Presentation Protocol Properties** dialog (using stacked spectra layout).

- **Background Margins:** these settings control the space between the spectra and the edge of the experiment.
- **Mass Labels:** these settings control where the mass labels are displayed relative to the edge of the experiment, and to the spectra. The **Upper** and **Lower** checkboxes determine if the labels are shown above or below the spectra.
- **Spectra Size:** controls the height and width of the spectra in the experiment.
- **Background:** this combo-box selects the background color for the experiment.
- **Layout:** selects from the three spectrum layouts — **Stacked**, **Overlaid**, or **Offset Overlaid** (see “Spectra layouts” on pages 87–89 for detailed information).



NOTE: Each of the three layouts retains its own margin, label, and spectra size settings — for example, changing the settings for spectra using **Offset Overlaid** will not affect the settings for spectra using **Stacked**, etc.

- **Display Tags:** controls whether the text to the right of the spectrum is displayed.
- **Use Margins:** determines whether the margins are used in calculating the distance between the page edge and the spectra.
- **Display Borders:** determines if a border is drawn around each spectrum. If deselected (i.e., if the borders are not displayed), it also changes the spectra background color to the experiment background color.
- **Space Between Spectra:** controls the amount of space between each spectrum in **Stacked** layout.

Offset Overlay options

When the display layout style is set to **Offset Overlay**, the **Experiment** page changes to include controls for adjusting the offset overlay.

- **X Offset:** the distance between spectra in the X dimension.
- **Y Offset:** the distance between spectra in the Y dimension.
- **Orientation:** determines the layout order— right to left, or left to right.

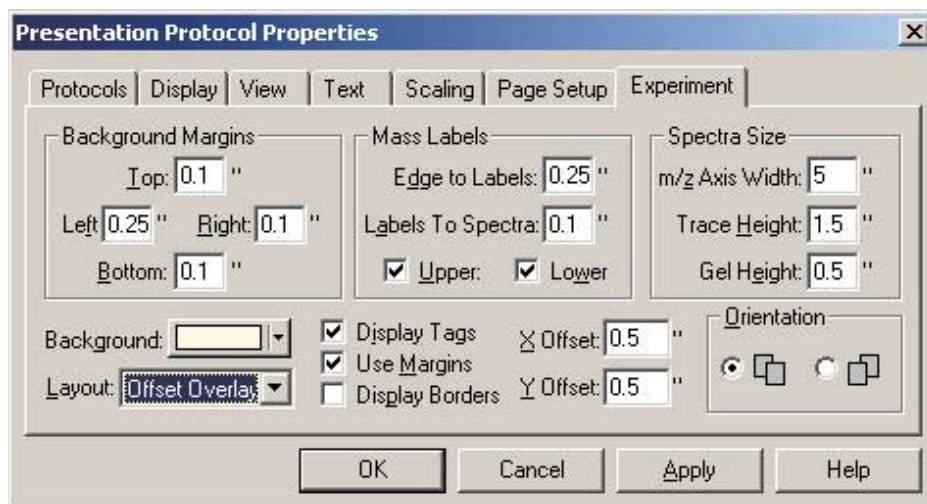


Figure 6-8: The **Experiment** page of the **Presentation Protocol Properties** dialog (using **Offset Overlay** layout).

Annotations

Annotations are used to add text comments to a spectrum or experiment. To add an annotation, select **Add Annotation** from the **Spectrum** menu or the **Experiment** menu. A frame will appear, in which you can enter text, and a formatting bar that controls the font and other attributes of the annotation.

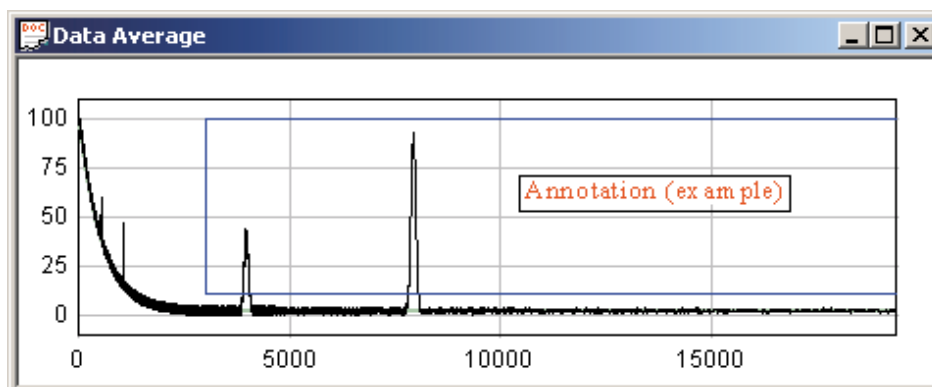


Figure 6-9: A spectrum with annotation.

To resize the annotation, move the cursor over the annotation frame to make the resizing handles appear. The handles are small black rectangles that will appear at the corners and middle of the borders of the annotation frame. Select a handle and drag it to resize the frame.

To move the annotation, move the mouse over the border of the annotation but not on a resizing handle, then click and drag it. Note that annotation positions are relative to the spectrum window, not to the mass or intensity scale.

Annotations can be hidden or displayed using the **Show/Hide Annotations** command in the **Spectrum** menu.

Reference lines



Reference lines (Figure 6-10) allow identification of multiply charged peaks by visual inspection. To display reference lines, select **Reference Lines...** from the **View** menu or click the **Reference Lines** toolbar button. (Note: the **Reference Lines** button is not installed in the toolbars by default. To use it, you will need to use the ProteinChip Software's **Customize** function to add it to the toolbar of your choice. See "Customizing toolbars" on page 128 for information on adding buttons to toolbars.)

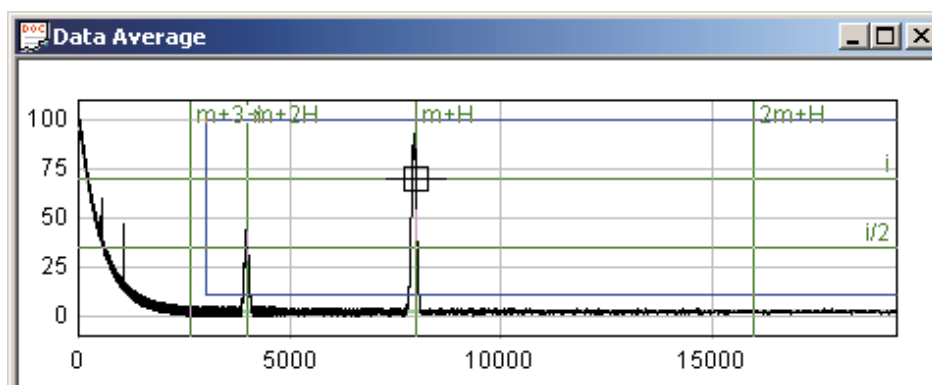


Figure 6-10: A spectrum with reference lines.

Many of the reference line features can be customized in the **Reference Lines** dialog (Figure 6-11), including the line colors, fonts and where the lines are drawn. The dialog is accessed by selecting **Reference Lines...** from the **Options** menu.

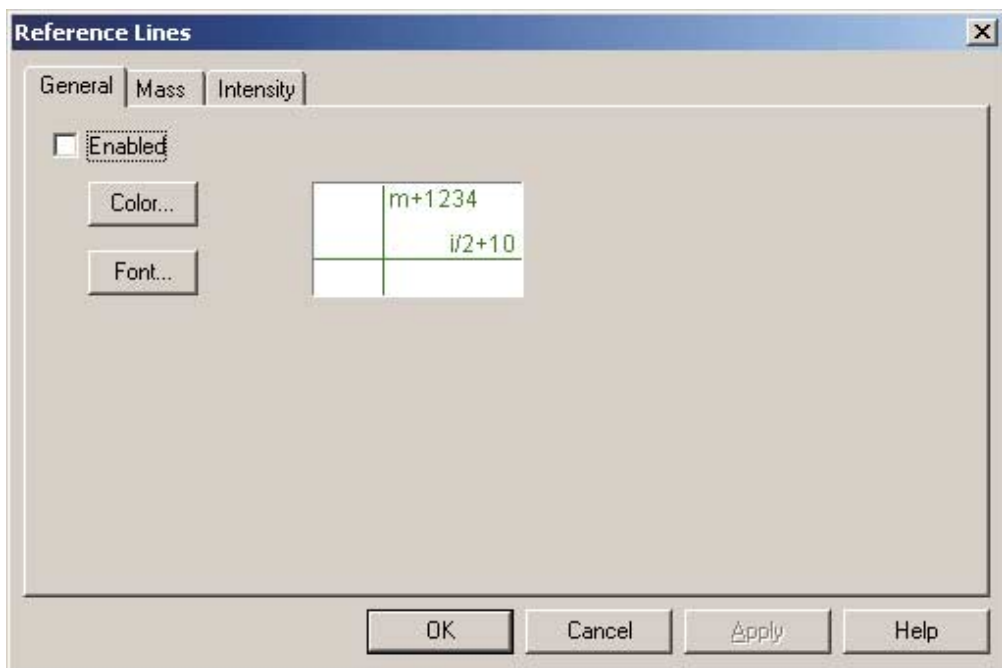


Figure 6-11: The **General** page of the **Reference Lines** dialog.

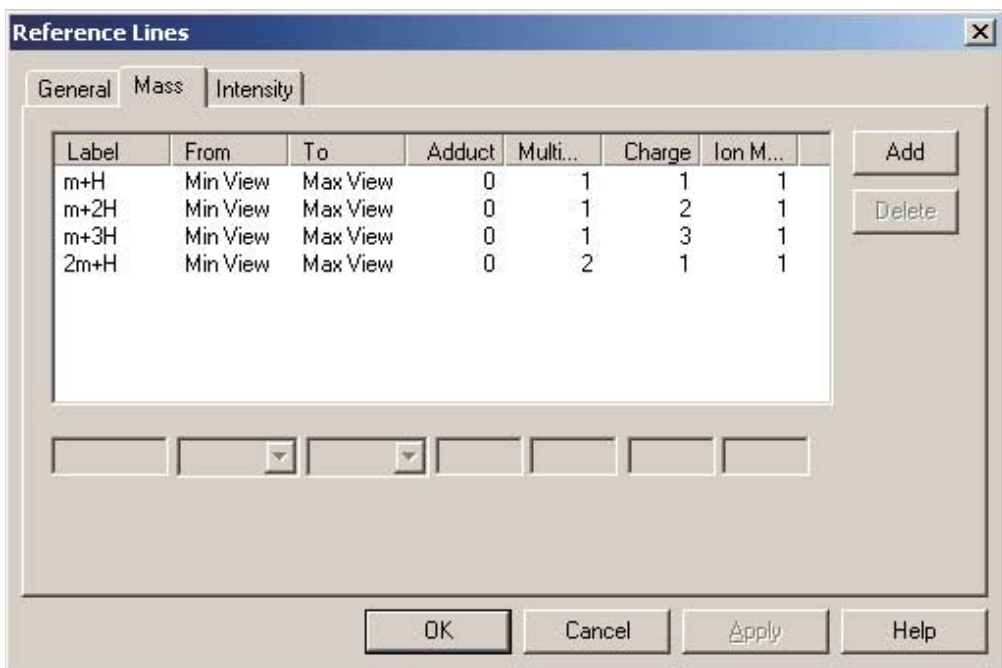


Figure 6-12: The **Mass** page of the **Reference Lines** dialog.

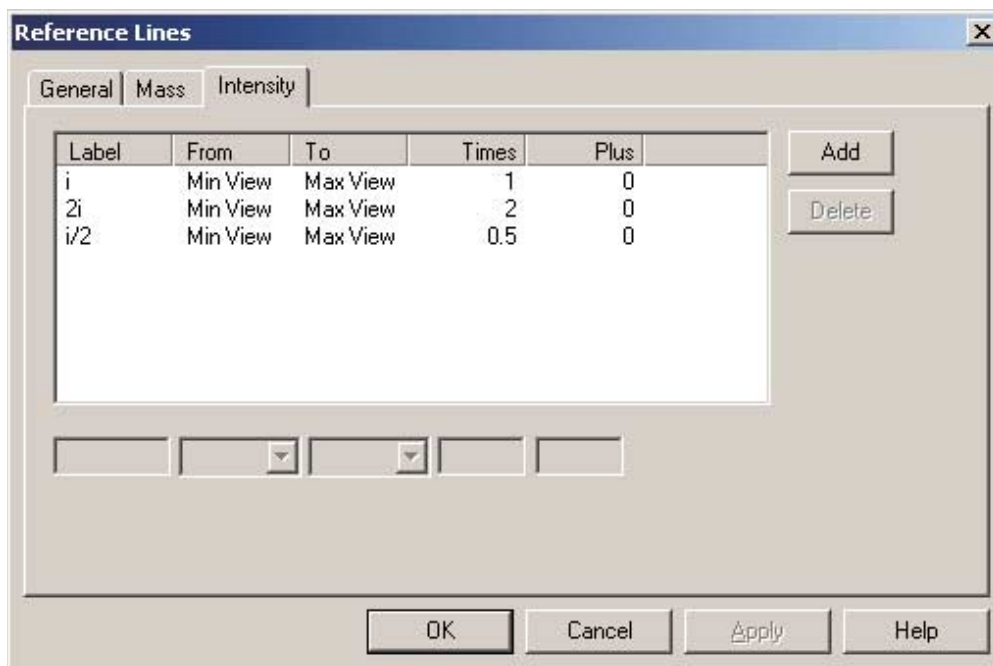


Figure 6-13: The *Intensity* page of the *Reference Lines* dialog.

Listing substances

The **List Substances** function is used to display a list of all labelled peaks in a spectrum. To access the display, select **List Substances...** from the **Peaks** menu. The table of peak information will be displayed in a separate window.

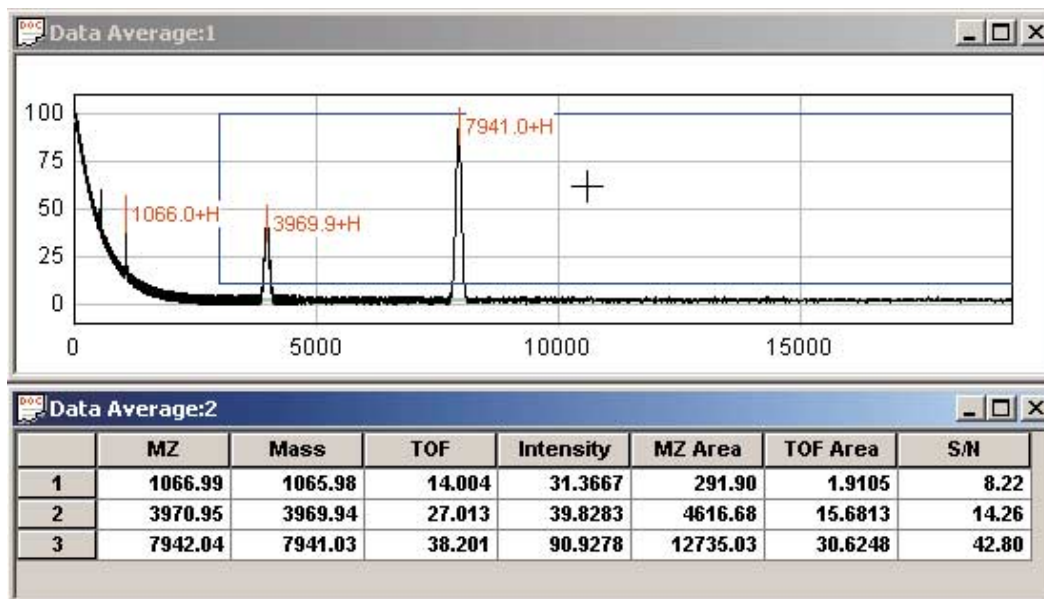


Figure 6-14: A spectrum and its tabular peak listing.

To copy values from the table to the Clipboard, select the rows or columns of data to copy and press Ctrl-C.

To print the table, click the **Print** toolbar button or select **Print...** from the **File** menu.

Customizing peak labels

Peak labels can be customized to display various peak parameters, or to display custom text by using the **Peak Label** dialog. Individual peak labels can be customized, or all labels on a selected spectrum can have the same labeling format applied to them.

To access the **Peak Label** dialog:

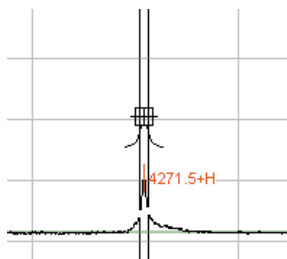


Figure 6-15: The **Centroid** cursor.

2. Move the cursor over a labeled peak.
3. Right-click the mouse and select **Peak Label** from the pop-up menu to open the **Peak Label** dialog.

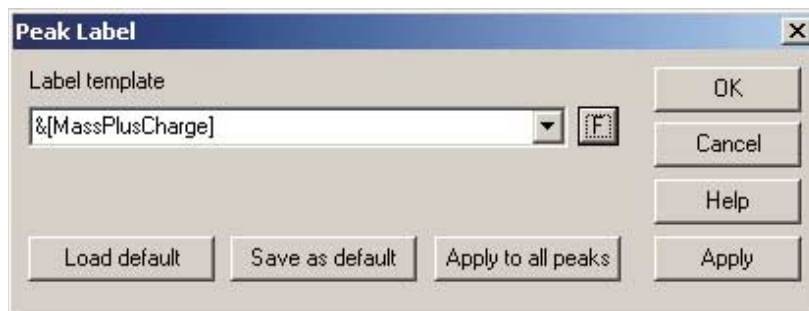


Figure 6-16: The **Peak Label** dialog.

- **Label template:** the text in this field controls how the peak is labeled. The text inside the symbols **&[]** will be replaced by the software with a measured peak parameter. To use literal text as a peak label, add text outside of the special **&[]** characters.

- **F:** this button displays a list of template values such as **Mass**, **SignalToNoise** and other values that will be automatically replaced with measured values from the peak.
- **Load default:** loads the default peak label template into the **Peak Label** dialog.
- **Save as default:** saves the current template as the new default peak label template for the current user.
- **Apply to all peaks:** applies the current peak label template to all of the peaks in the selected spectrum.

Appendix A: Windows and Toolbars

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This appendix contains a brief introduction to the names and organization of the program windows and toolbars. The remainder of the manual includes more detailed descriptions of the program functions.

The main window

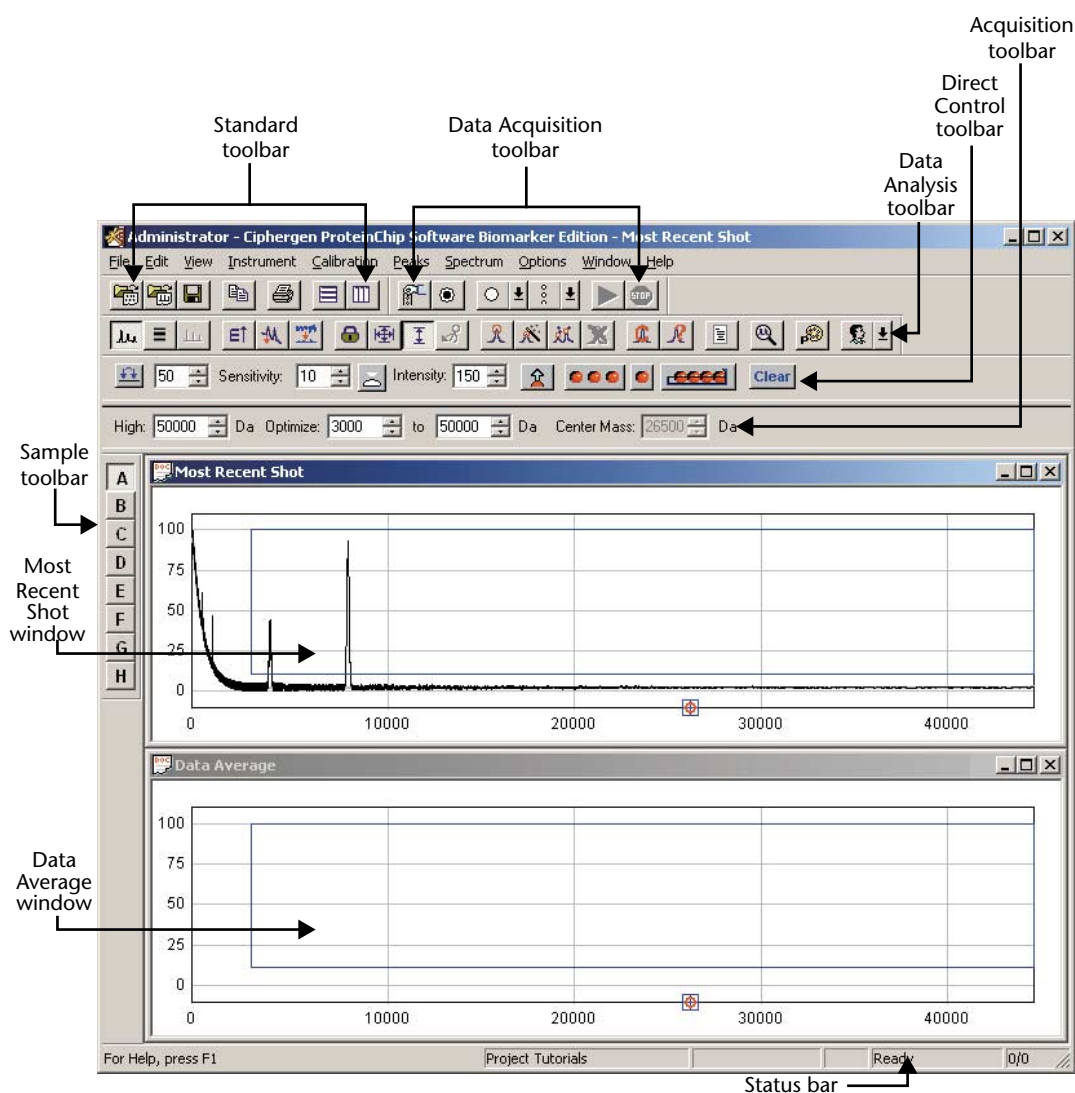


Figure A-1: The main window of the ProteinChip Software.

- The **Standard** toolbar contains controls for working with files, allowing you to open, save, print, and export data from the files.
- The **Data Acquisition** toolbar contains controls for selecting protocols and starting and stopping spot and chip protocols.

- The **Data Analysis** toolbar contains controls for common analysis tasks. These controls affect only the way the data is displayed — they have no effect on the underlying data.
- The **Direct Control** toolbar includes controls for the laser, and other controls used during manual data collection.
- The **Acquisition** toolbar controls the mass range to acquire, mass optimization range and the focus mass if the instrument is equipped with time lag focusing.
- The **Sample** toolbar controls and displays which sample spot is being analyzed.
- The **Most Recent Shot** window shows the result of the last laser shot.
- The **Data Average** window stores the sum of the transients accepted and added to the average.
- The **Status** bar displays the instrument status and the current project.

The Standard toolbar

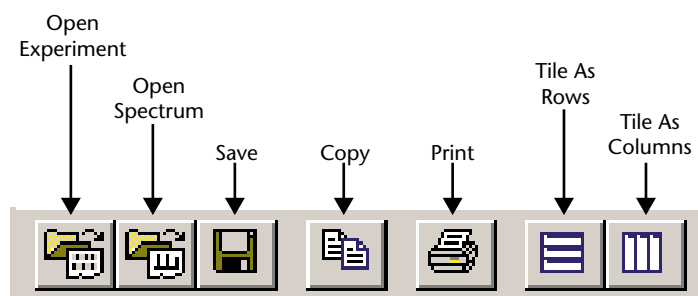


Figure A-2: The *Standard* toolbar.

The **Standard** toolbar contains controls for working with files, allowing you to open, save, print, and export data from the files.

- **Open Experiment:** opens an experiment file from the list of previously saved experiments in the selected project. The experiment will be opened in a small window in the main pane of the ProteinChip Software, and the Experiment toolbar will be displayed at the top of the experiment (see “*The Experiment toolbar*” on page 126).
- **Open Spectrum:** allows selection of individual spectra from the list of all spectra in the current project.
- **Save:** saves the selected spectrum or experiment data file.
- **Copy:** copies selected spectra to the clipboard. The copied spectra can then be pasted as data into an experiment, or as a graphic element into a document.
- **Print:** prints the selected spectra or experiment.
- **Tile as Rows:** arranges the displayed items as equally-sized windows in row format (horizontal).

- **Tile as Columns:** arranges the displayed items as equally-sized windows in column format (vertical).

The Data Acquisition toolbar

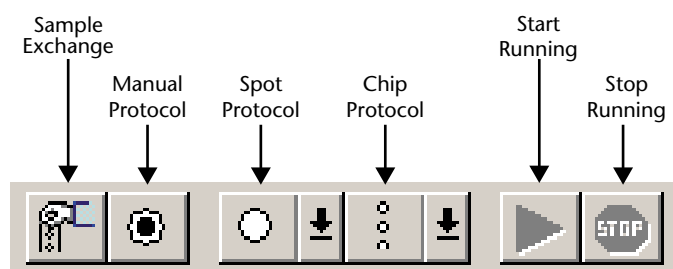


Figure A-3: The Data Acquisition toolbar.

The **Data Acquisition** toolbar contains controls for selecting protocols and starting and stopping spot and chip protocols.

- **Sample Exchange:** shows or hides the **Sample Exchange** dialog.
- **Manual Protocol:** opens the **Manual Protocol** dialog.
- **Spot Protocol:** opens the spot protocol wizard or selects from a list of existing spot protocols.
- **Chip Protocol:** opens the chip protocol wizard or selects from a list of existing chip protocols.
- **Start Running:** starts the selected spot or chip protocol.
- **Stop Running:** cancels the execution of a running protocol, or stops manual data acquisition.

The Data Analysis toolbar

The **Data Analysis** toolbar contains controls for common data analysis tasks. These controls affect only the way the data is displayed, and have no effect on the underlying raw data.

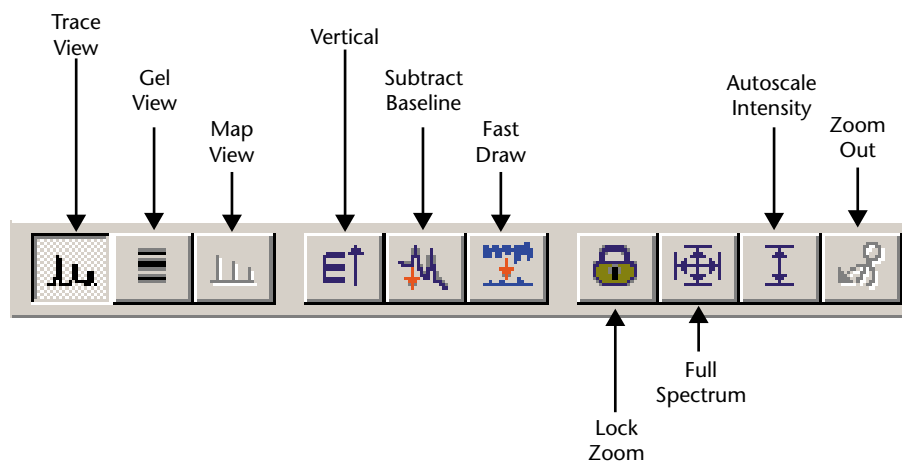


Figure A-4: The *Data Analysis* toolbar, part 1.

- **Trace View:** displays data as peaks in spectra.
- **Gel View:** displays data as bands, similar to gel electrophoresis.
- **Map View:** peaks are displayed as thin vertical lines.
- **Vertical:** changes the mass axis to vertical.
- **Subtract Baseline:** subtracts the baseline from the displayed spectrum.
- **Fast Draw:** speeds up redrawing of the spectra by averaging the data points that make up the displayed trace.
- **Lock Zoom:** forces all displayed spectra to show the same data range.
- **Full Spectrum:** zooms all the way out.
- **Autoscale Intensity:** when selected, the displayed data is automatically adjusted to the maximum dynamic range.
- **Zoom Out:** zooms out in stepwise fashion.

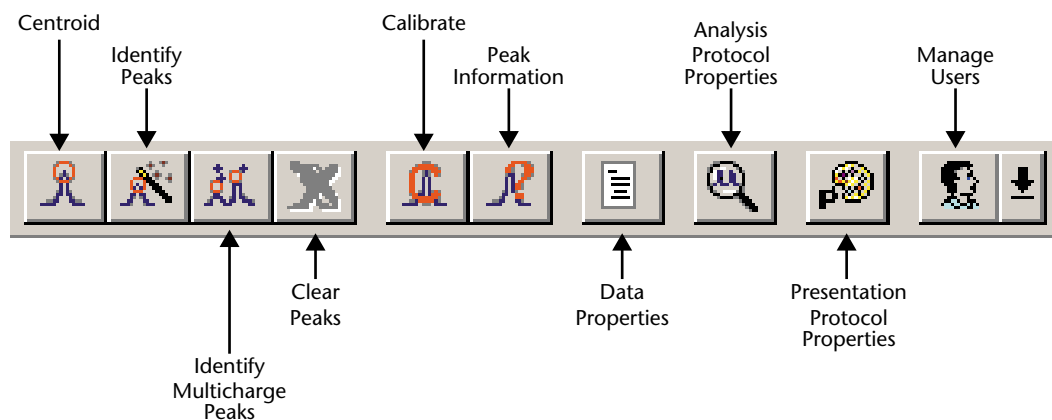
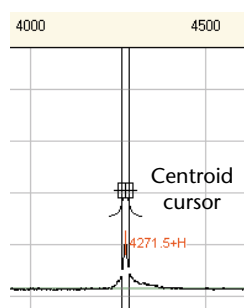


Figure A-5: The *Data Analysis* toolbar, part 2.

- **Centroid:** activates the centroid cursor that is used to identify a region in the display as a peak (or band, in gel view). Only “centroided” peaks or bands are visible in map view.



*Figure A-6: Clicking the **Centroid** button changes the cursor to the centroid cursor.*

- **Identify Peaks:** automatically labels peaks in the spectrum according to specified criteria.
- **Identify Multicharge Peaks:** identifies potential multiply-charged peaks ($Z > 1$).
- **Clear Peaks:** removes peak labels (centroids) from the displayed portion of the spectrum.
- **Calibrate:** changes the cursor to the calibration cursor, and opens the **Calibration** dialog box.
- **Peak Information:** sets the cursor to peak information mode — click on any peak or band to open the **Peak Information** dialog.
- **Data Properties:** displays detailed information about the selected spectrum, including the instrument settings and the spot protocol.
- **Analysis Protocol Properties:** opens the **Analysis Protocol Properties** dialog, containing settings for peak detection, baseline subtraction, noise, filtering, etc.
- **Presentation Protocol Properties:** opens the **Presentation Protocol Properties** dialog, which controls how the experiment or spectrum is displayed on-screen and how it is printed.
- **Manage Users:** opens the **User Manager** dialog.

The Direct Control toolbar

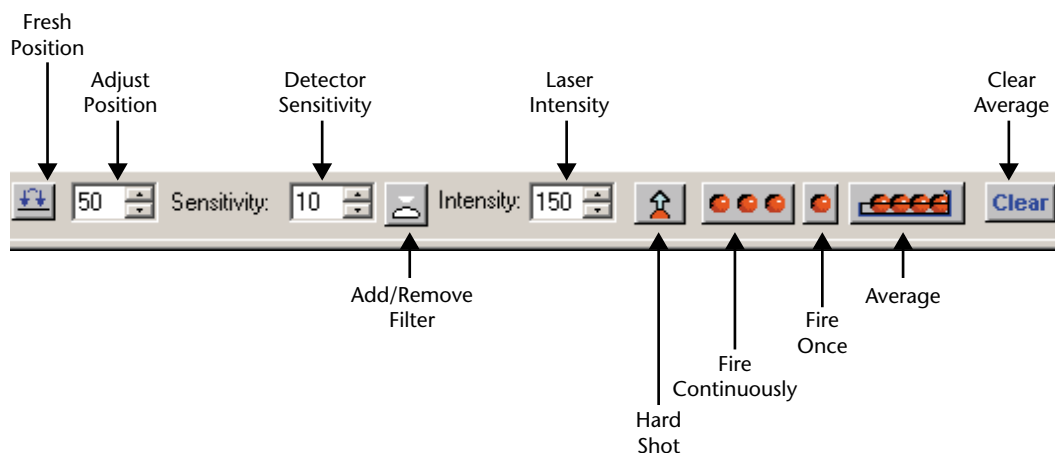


Figure A-7: The *Direct Control* toolbar.

The **Direct Control** toolbar includes controls for the laser, and other controls used during manual data collection.

- **Fresh Position:** moves the laser to a new position on the spot.
- **Adjust Position:** controls the location of the laser on the sample spot. Position 50 is the center of the spot; normally the position should be kept between 20 and 80.
- **Detector Sensitivity:** determines the amplification of the digitizer. Adjust the value higher if no peaks are visible and lower if peaks of interest are off scale.
- **Add/Remove Filter:** controls the filter placement: in (less light) or out (more light). This feature is present only on the model PBS I reader.
- **Laser Intensity:** controls the amount of laser light that ionizes the sample.
- **Hard Shot:** boosts the laser energy by a specified amount to warm sample positions. This can be helpful when moving to a new sample location.
- **Fire Continuously:** fires the laser repeatedly until the button is clicked again.
- **Fire Once:** fires the laser once.
- **Average:** adds the transient in the **Most Recent Shot** window to the data average.
- **Clear Average:** clears all data from the **Data Average** window.

The Acquisition toolbar

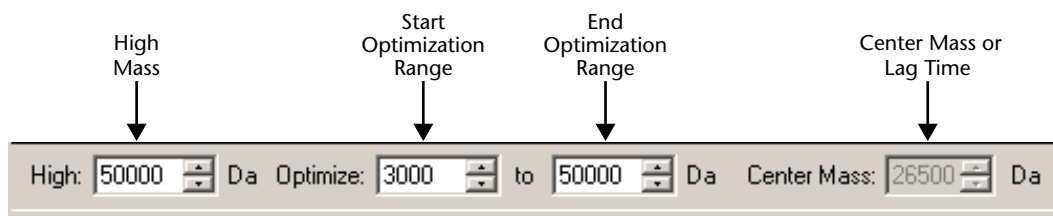


Figure A-8: The Acquisition toolbar.

The **Acquisition** toolbar controls the mass range to acquire, mass optimization range and the focus mass if the instrument is equipped with time lag focusing.

- **High Mass:** specifies the highest mass to collect. Typically, the value is set larger than twice the mass of the highest mass peak of interest.
- **Start Optimization Range:** sets the low mass for the optimization range.
- **End Optimization Range:** sets the high mass for the optimization range.
- **Center Mass or Lag Time:** controls the time lag focus settings on instruments equipped with time lag focusing capabilities. The function and label change depending on the pulse-focusing mode.

The Sample toolbar

The **Sample** toolbar selects the sample spot to analyze.



Figure A-9: The Sample toolbar.

The Experiment toolbar

The **Experiment** toolbar is anchored to the experiment window frame. It contains the controls for some of the most common experiment operations.

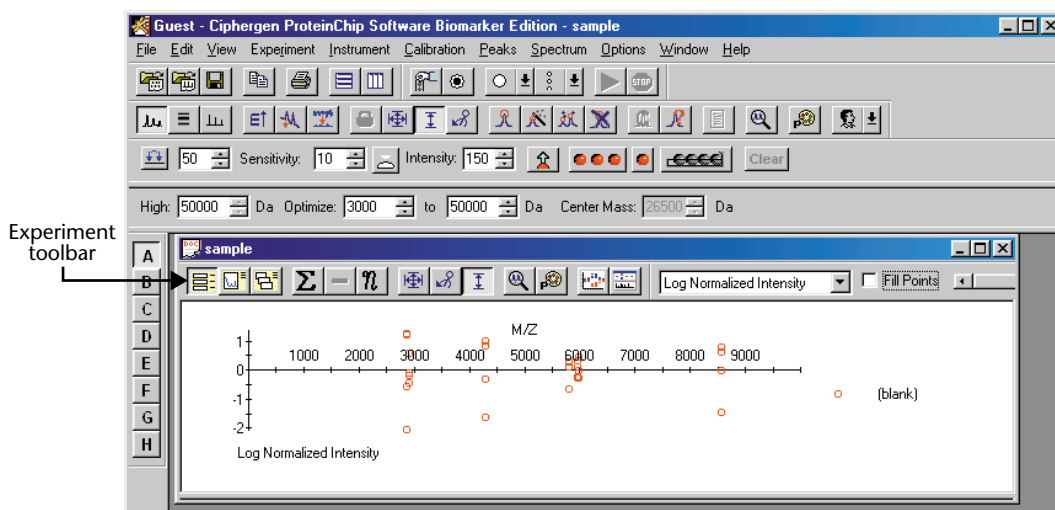


Figure A-10: The **Experiment** toolbar is anchored to the top of the experiment window.

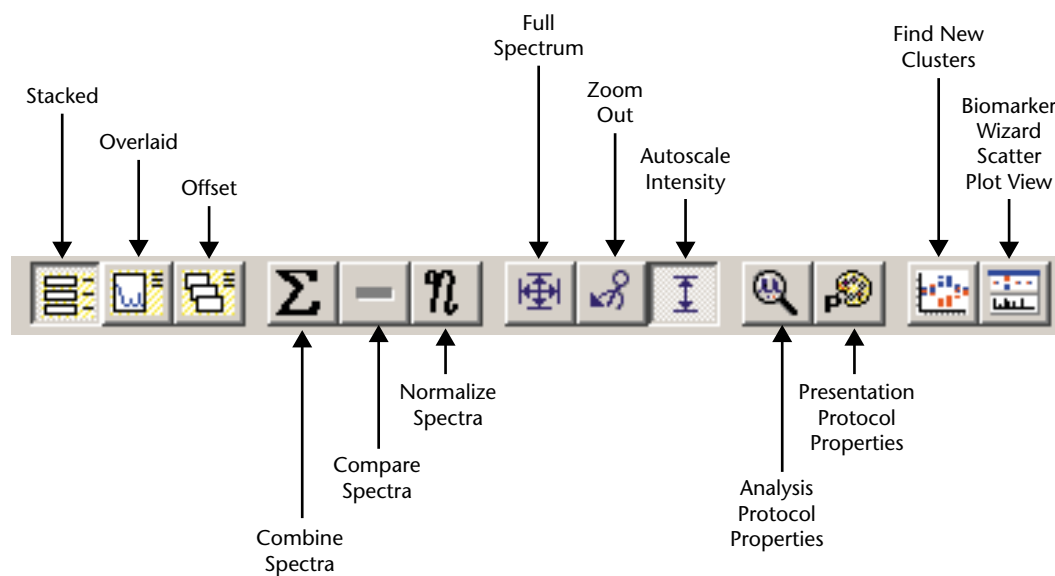


Figure A-11: The **Experiment** toolbar.

- **Stacked:** spectra are displayed next to each other.
- **Overlaid:** spectra are overlaid directly on top of each other.
- **Offset:** spectra are overlaid with a slight offset.
- **Combine Spectra:** combines 2 or more spectra into a peak map.
- **Compare Spectra:** compares two spectra or peak maps.

- **Normalize Spectra:** normalizes peaks over multiple spectra.
- **Full Spectrum:** displays the complete spectrum.
- **Zoom Out:** zooms out in a stepwise manner.
- **Autoscale Intensity:** autoscales the displayed spectrum to the full intensity range.
- **Analysis Protocol Properties:** opens the **Analysis Protocol Properties** dialog, containing settings for peak detection, baseline subtraction, noise, filtering, etc.
- **Presentation Protocol Properties:** opens the **Presentation Protocol Properties** dialog, which controls how the experiment or spectrum is displayed on screen and how it is printed.
- **Find New Clusters:** opens the **Biomarker Wizard – Generate New Clusters** dialog.
- **Biomarker Wizard Scatter Plot View:** toggles between displaying the Biomarker Wizard scatter plot and the raw data.

To the right of the **Biomarker Wizard Scatter Plot View** button are the **View** drop-down menu, containing the display options for Biomarker Wizard scatter plots, and the **Fill Points** checkbox (Figure A-12). These items are only active when a Biomarker Wizard scatter plot is displayed in the **Experiment** window. See “*Viewing Biomarker Wizard plots*” on page 99 for more information on using these features.

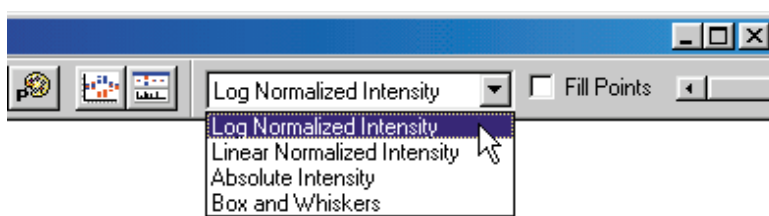


Figure A-12: The **View** drop-down menu and the **Fill Points** checkbox.

Customizing toolbars

You can change the arrangement of the buttons in the toolbars. To do so, choose **View | Toolbars | Customize** to open the **Customize** dialog.

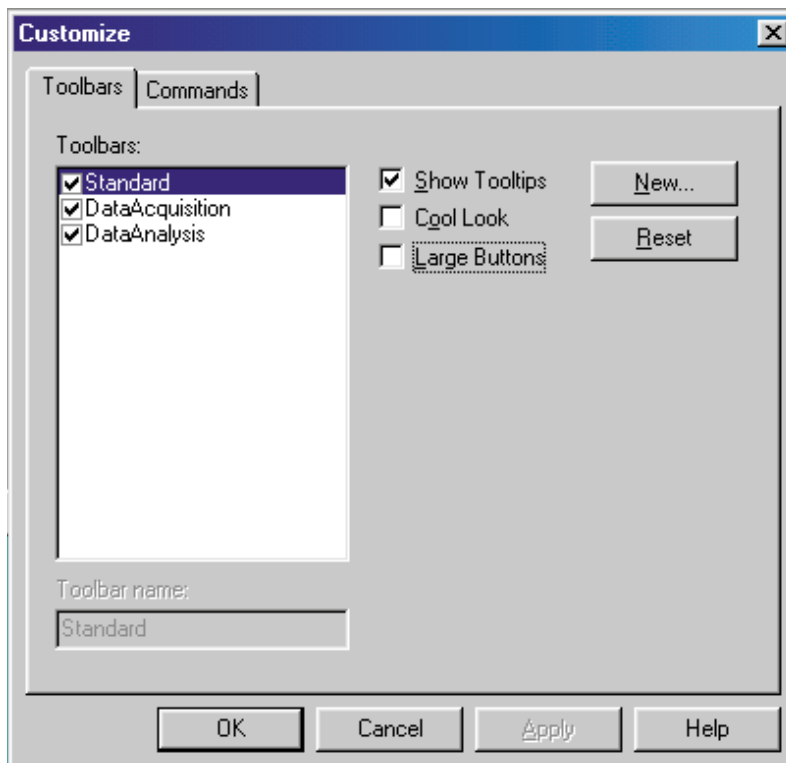


Figure A-13: The Toolbars page of the Customize dialog.

The dialog opens to the **Toolbars** page, which allows you to toggle the display of the **Standard**, **Data Acquisition**, and **Data Analysis** toolbars, as well as to choose to show Tooltips for them.

The second page of the **Customize** dialog is the **Commands** page, which allows you to customize the arrangement of the toolbar buttons. Select a category from the drop-down list to display the buttons in that category. Clicking a button will select the button and display its description in the **Description** field.

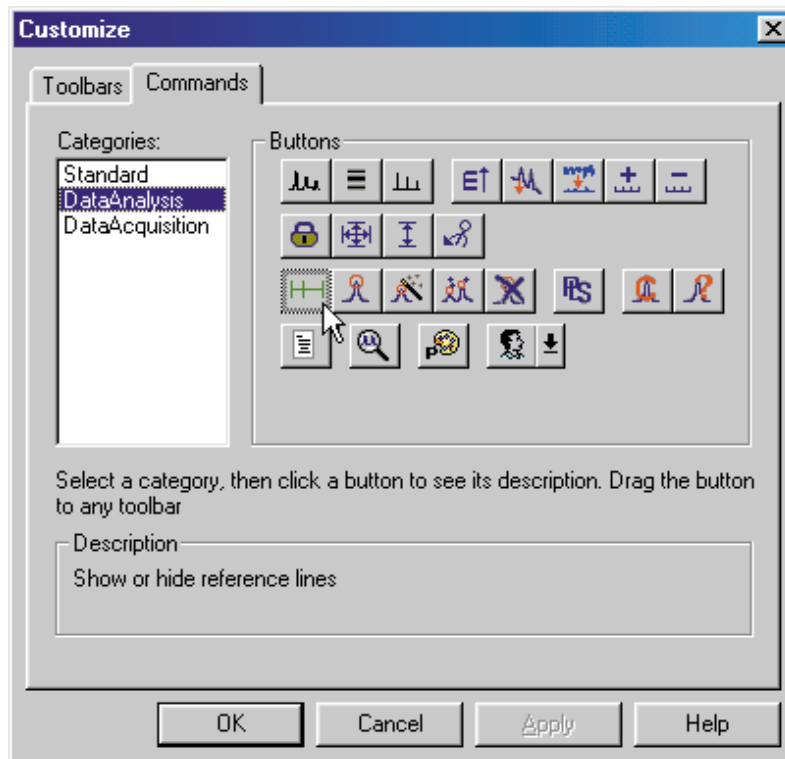


Figure A-14: The Commands page of the Customize dialog.

To move a button to a new toolbar location, select it in the **Buttons** section of the dialog to make it appear on-screen, then drag and drop the button into desired location on the toolbar of your choosing.

Appendix B: New in Version 3.0

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This appendix summarizes the changes between the previous version of ProteinChip software, 2.1 and version 3.0. This content will be of interest if you have used 2.1 to collect or manage ProteinChip data in the past, but can be safely ignored if you are new to using the ProteinChip software.

Databases

Many of the enhancements to this version are enabled by using a database to store important information about the data. The database allows the software to build new experiments from existing spectra, and to sort and manage the spectra properties. These properties include the sample information associated with each spectra along with all of the ProteinChip® protocol conditions such as the type of ProteinChip array, the wash conditions and the data collection parameters. This arrangement makes it easy to construct experiments for the conditions that you wish to analyze, and will allow much greater flexibility with data management in future versions. See Chapter 2, *“Managing Data and Users”* for details about how to manage data using the database.

Biomarker Wizard

The Biomarker Wizard has been significantly enhanced. Important changes include:

- The output of the Biomarker Wizard is now in a splitter window. The mass range of the plot tracks the that of experiment.
- A box and whisker plot is available to summarize complex biomarker patterns.
- The clustering function now allows the use of manual peaks. This allows you to define the peaks of interest in a few spectra and then allow Biomarker Wizard to find or estimate the peaks for the remaining spectra.
- Peak clusters can be saved and reloaded to another experiment. This allows you to compare the same markers across multiple experiments.
- Biomarker Wizard can export data in a format compatible with Biomarker Patterns Software.

See Chapter 5, *“Experiments”* for details about using Biomarker Wizard.

Normalization

A new option in the **Normalization** dialog allows normalizing to total ion current. This method generally reduces the average variation of spectra across an experiment, and is the recommended default for expression profiling. See *“Normalizing spectra”* in Chapter 5, *“Experiments”* for details.

Signal enhancement

A new option in the **Filtering** page of the **Analysis Protocols Properties** dialog enables a variable gain setting for high mass plus an additional filtering step. The combination improves the signal to noise ratio of high mass peaks. To use the feature, check the **Signal Enhance** checkbox on the **Filtering** page (for more information, see *"The Filtering page"* on page 66).

Mass calibration equation

The form of the mass calibration has been changed from a cubic to a quadratic fit to allow calculating the high order coefficients with fewer calibration peaks. The mass accuracy of internal calibrations can be greatly improved over the linear defaults that the previous algorithm used.

Another significant improvement to the mass calibration is the way in which calibrations are adjusted when using a single point calibration. Previous versions of the software required at least two points for an accurate mass calibration whereas this version will generate good calibrations with even a single internal standard.

Calibration protocols

Calibration protocols automate the process of mass calibration. The protocols contain a list of mass calibrants and rules that allow the software to identify the calibration peaks and generate a new mass calibration. This makes utilizing a mass standard such as the All-in-1 Peptide Standard mix much easier. In addition, the calibration protocols can be applied to multiple spectra in an experiment, allowing you to align the mass of common peaks in a series of spectra from a protein profiling experiment.

Calibration equations

The software now allows saving a calibration equation from a spectrum and applying calibration equations to one or more spectra.

See Chapter 3, *"Calibration"* for more details about using the calibration equations.

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